UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

NOV 19 1998

OFFICE OF PREVENTION,
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: EPA Registration 070759-R; **INSECT REPELLENT 3535 (IR3535™)** (100% Ethyl-3-(N-butylacetamido)propionate), Insect Repellant; Product Chemistry, Acute and Chronic Toxicity and Efficacy Studies; MRID Nos. 442708-01 through -40, 442979-01 through -07 and 444824-01 (Chemical #113509; Case 061746; Submissions #S524885 and S538640; DP Barcodes D237154 and D244720).

FROM: Carol E. Frazer, Ph.D. Carol
Biochemical Pesticides Branch
Biopesticides and Pollution Prevention Division (7511C)

THROUGH: Roger Gardner, Toxicology Reviewer, Russell S. Jones, Ph.D., Efficacy Reviewer, Freshteh Toghrol, Ph.D., Chemistry Reviewer
Biochemical Pesticides Branch
Biopesticides and Pollution Prevention Division (7511C)

TO: Sheila Moats, Ph.D., Regulatory Action Leader
Biochemical Pesticides Branch
Biopesticides and Pollution Prevention Division (7511C)

ACTION REQUESTED:

Ms. Anne Gochman, agent for Merck KGaA of Darmstadt, Germany, submitted an application to register a new biochemical pesticide technical grade active ingredient on April 2, 1997. It included product chemistry, acute and chronic toxicologic and efficacy studies comprised of the §61, §62, §63 series and some of the §81 series: §81-1, 81-3, 81-4 and 81-6 as well as §82-3, §83-1, 83-3, §84, §85, §95-9 and §152-16. The foregoing studies are subsumed in MRID Nos., 442708-01 through 40, 442979-01 through 8 and 444824-01.

NOTOX Safety & Environmental Research B.V. performed chemistry testing on MRID numbers 442708-03 through -19 and 444824-01. Toxicologic studies were conducted as follows:

Merck (MRIDs 442708-20, -22, -26, -29, 442979-04, -07, -08), BASF (442708-21), WIL Research Laboratories (MRIDs 442708-25, -39, 443233-01), RCC (MRIDs 442708-33, -34, -35, 442979-03, -06), Corning-Hazleton (MRIDs 442708-30, -31), University of Mainz (MRID 442979-05), Florida A & M University (MRID 442708-36), Wildlife Research Station, Algonquin, Ontario and Trent University, Peterborough, Ontario (MRID 442708-37), Benzon Research (MRID 442708-38), and Hill Top (MRID 442979-02).

Supplemental studies were also submitted as follows: Chemistry Information (MRID 442979-01), primary irritation and dermal sensitization (MRID 442708-23), 4-Week Oral Toxicity (MRID 442708-24), Investigatory oral studies in rabbits (MRID 442708-27 and 28), the Two-week Oral Toxicity by Gavage in Rabbits (MRID 442708-39) and the Toxicology Summary and U.S. Army Hazard Evaluation (MRID 442708-32).

CONCLUSIONS:

BPB considers the material supplied by Merck KGaA insufficient to register their new biochemical pesticide technical grade active ingredient. The material supplied for §62-2 is incorrect. The limits given in the Certified Statement of Formulation (CSF) are not derived from §62-1 and do not match the value given on the label. These must be re-submitted before complete acceptance.

TOXICITY PROFILE

Acute oral toxicity	IV	Acceptable
Acute dermal toxicity	IV	Acceptable
Acute inhalation toxicity	IV	Acceptable
Primary eye irritation	II	Acceptable
Primary dermal irritation	IV	Acceptable
Dermal sensitization	No	Acceptable

LABELING: The Signal word is "Warning" because of primary eye irritation of grade II. The following labeling language is acceptable:

SIGNAL WORD: WARNING

PRECAUTIONARY STATEMENTS:

Causes substantial but temporary eye injury. Harmful if absorbed through skin. Do not get in eyes or on clothing. Avoid contact with skin. Wear goggles or face shield. Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash clothing before reuse.

STATEMENT OF PRACTICAL TREATMENT (SOPT):

IF SWALLOWED: Call a physician or Poison Control Center. Do not induce vomiting. Drink promptly a large quantity of milk, egg whites, gelatin solution, or if these are not available, drink large quantities of water. Avoid alcohol.

IF ON SKIN: Wash with plenty of soap and water.

IF IN EYES: Hold eyelids open and flush with steady, gentle stream of water for 15 minutes. Get medical attention.

BPB's reviews of data are summarized below.

Study Summaries:

PRODUCT CHEMISTRY

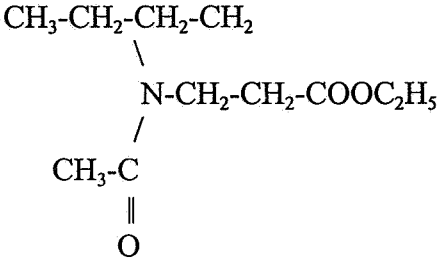
Guideline §61-1: Product identity and disclosure of ingredients (Label and MRID 442708-01)

INSECT REPELLENT 3535 (IR3535™) contains 100% (ethyl-3-(N-butylacetamido) propionate), a substituted amino acid structurally similar to β-alanine, a naturally occurring β amino acid. This product is to be used to formulate end-use insect repellents against mosquitos, deer ticks, body lice and biting flies.

The following table summarizes information submitted by the registrant regarding the active ingredient.

Chemical Name:	[N,n-Butyl-N-acetyl]aminoproprionic acid-ethyl ester
CAS Registry No.:	52304-36-6
Synonyms:	Ethyl-3-(N-butylacetoamido)propionate β-alanine, N-acetyl-N-butyl-, ethyl ester 3-(N-butylacetamido)propionic acid ethyl ester 3-[N-n-butyl-N-acetyl]-aminopropionic acid-ethylester $C_{11}H_{21}NO_3$
Chemical Family:	Substituted amino acid (alanine)
Source of Biochemical	Manufactured
Mode of Action:	Repellant

Chemical structure:



This compound has been used in Europe as an insect repellent for more than 20 years. It is a substituted β amino acid, similar to β-alanine.

An acceptable confidential statement of formula was submitted by the registrant.

BPB's Comment: Data submitted on the product identity satisfy the requirements of 40 CFR 158.690.

Guideline §§61-2(a) and (b): Manufacturing process (MRID 442708-02)

In Confidential Appendix

Guideline §61-3: Discussion on the formation of unintentional ingredients (MRID 442708-02; MRID 442979-01)

In Confidential Appendix

Guideline §62-1: Analysis of samples (MRID 442708-03)

In Confidential Appendix

Guideline §62-2: Certification of ingredient limits (MRID 442708-03)

In Confidential Appendix

Guideline §62-3: Analytical methods for certified limits (MRID 442708-03; MRID 442979-01)

In Confidential Appendix

Guideline §63: Physical and Chemical Characteristics (MRIDs 442708-06 through -19)

The registrant submitted information on the physical and chemical characteristics of the formulated end-use product which are summarized below:

Study Type	Characteristic	Source
Color	Colorless	MRID 442708-04
	Clear, colorless to slightly yellowish	MRID 442979-01
Physical State	Liquid at room temperature	MRID 442708-04
Odor	None	MRID 442708-04
	Almost odorless	MRID 442979-01
Boiling Point (estimated)	Slightly below 300 °C at ambient pressure	MRID 442708-05
Density	0.998 g/cm^3 at 20.0 ± 0.5 °C	MRID 442708-06
Solubility	Water at 20.0 ± 0.5 °C (mean): 70 g/l	MRID 442708-07
(Organic Solvents)	Acetone: > 1000 g/l	MRID 442708-08

	Ethylacetate: > 1000 g/l Dichloromethane: > 1000 g/l n-Heptane: > 1000 g/l Methanol: > 865 g/l *p*-Xylene: > 1000 g/l	
Vapor Pressure	p (20°C) = 0.15 ±0.01 Pa = (11.2 ± 0.8) x 10^{-4} mm Hg	MRID 442708-09
n-Octanol/Water	54 (log P_{OW} = 1.7) at 23-24°C	MRID 442708-10
pH	4.882 ± 0.021 (95% confidence interval; n=5) 1% (w/v) aqueous dispersion at 20.0 ± 0.1°C	MRID 442708-11
Stability	0.0 ± 0.1 °C (1 hr, 7 days): no visual consistency changes	MRID 442708-12
	54 ± 2°C (14 days): purity (g/kg) unchanged	MRID 442708-13
	Direct photolysis in water (8 days): no change in HPLC	MRID 442708-14
	copper/iron and copper/iron ions in 20% ethanol (14 days): No change in concentration or appearance	MRID 442708-15
Flash-point	159°C, 318°F	MRID 442708-16
Storage stability	No significant change in active ingredient as indicated by new peaks in GC chromatograms at 0, 3, 6, 7, 9, 12 months, in concentration (100→97.4% at 12 months) or in visual appearance of the commercial container (high density polyethylene [HDPE]) after one year in storage at ambient temperature (4.5→23.5°C) and humidity (45→87%)	MRID 442708-17, 444824-01
Viscosity	14 mPas at 20 ± 0.5°C, 16 mPas at 40 ± 0.4°C	MRID 442708-18
Corrosion characteristics	The copper/iron study covered in Stability above, includes a statement that these are "...the metals and solutions that IR3535 will come in contact with during its storage and use." The storage stability study also covered the corrosion characteristics of the HDPE storage container for 12 months. No change in appearance was observed.	MRIDs 442708-15, -17, 444824-01

PRODUCT TOXICOLOGY

Guideline §81-1: Acute Oral Toxicity Study in Rats (MRID 442708-20) A limit dose of IR3535 (5,000 mg/kg) was tested by gavage in male and female rats. The LD_{50} was determined to be greater than 5,000 mg/kg. Classification: Acceptable; Toxicity Category IV.

Guideline §81-3: Acute Inhalation Toxicity Study in Rats (MRID 442708-21) A single dose of IR3535 was tested in male and female rats. The LC_{50} was determined to be greater than the concentration tested (>5.1 mg/l). Classification: Acceptable; Toxicity Category IV.

Guideline §81-4: Primary Eye Irritation Study in Rabbits (MRID 442708-22) Single (0.5 ml) dose applied to male and female rabbits' eyes. This substance is substantially irritating to rabbit

eyes, causing hyperemia, chemosis, discharge and corneal opacity in all animals, and, in one rabbit, the corneal effect lasted through day 7. Classification: Acceptable; Toxicity Category II.

Guideline §81-6: Delayed Contact Hypersensitivity in Guinea Pigs (Buehler Technique) (MRID 442979-02) Inducted and challenged with 0.3 ml of 100% test substance, no positive response. Positive control efficacious. Classification: Acceptable; not a dermal sensitizer.

Guideline §152-16: Consumer Information Concerning the Use of IR3535 in Product Formulations (MRID 442979-08) Of 13 companies who responded, only 1 reported any complaints about a product containing this chemical. But it was not specifically mentioned as to whether it was skin sensitization or irritation.

Guideline §82-3: Subchronic (90-Day) Dermal Toxicity Study in Rats (MRID 442979-03) In a study on IR3535 in Wistar rats at doses of 0, 100, 1,000 and 3,000 mg/kg/day, in which no dermal toxicity was noted, a NOEL of 3,000 mg/kg/day was assigned. Animals treated daily for 6 hours. Minimal clinical symptoms noted. Animals tested for hematology, clinical chemistry and ophthalmology and followed for a 2-week recovery period. Classification: Acceptable; assigned Toxicity Category IV for use as an Acute Dermal Toxicity Category, and IV for use in Primary Dermal Irritation Category

Guideline §83-3: Developmental Toxicity Study in Rabbits (MRIDs 442708-26, 443233-01) Study in Himalayan rabbits at ~100, 300 and 1,000 mg/kg/day. Maternal toxicity at all dose levels (reduced food consumption, deaths), no embryo or fetal toxicity at any dose. Classification: Unacceptable. A second study with New Zealand Whites at 100, 300, 600 mg/kg/day showed maternal toxicity at highest dose (effects on food consumption, body weight gain), no embryo or fetal toxicity at any dose. Classification: Acceptable, not a developmental toxicant

Guideline §83-4: 2-Generation Reproductive Study in Rats (MRID 442979-04) Doses of 100, 300 and 1,000 mg/kg/day resulted in increased liver and kidney weights at the mid and high dose groups of both sexes for parents, and for kidneys of the high dose group in second generation. Changes in adrenals and spleen observed, but restricted to one dose group or sex. No effects on reproductive parameters, or other physiologic changes in parents or offspring of either generation. Classification: Acceptable, not a reproductive toxicant

Guideline §84-2: Mutagenicity Studies in Bacteria, Mammalian cells *in vitro* and Mouse Bone Marrow (MRIDs 442708-29, 30, 31, 442979-05) Several strains of *Salmonella typhimurium* and *Escherichia coli* were used to test for mutagenicity of IR3535 up to 5,000 µg/plate, both with and without activation. No positive responses. Gene mutation in V79 cells up to 5,000 µg/ml was not seen, with or without activation. Chromosomal aberrations in Chinese hamster ovary cells at up to 5,000 µg/ml of IR3535 was ineffective, with or without activation, except at cytotoxic levels. Examination of micronuclei in mouse bone marrow following treatment of IR3535 showed no effects at doses of 5,000 mg/ml. All studies included appropriate positive controls, which were efficacious. Classification: Acceptable, neither a genetic nor a chromosomal mutagen.

Guideline §85-1, 3: Metabolism. Pharmacokinetic Studies in Rats, Rabbits and Man (MRIDs 442708-33, 34, 35, 442979-06, 07) Several metabolism, pharmacokinetic, toxicokinetic, bioretention, elimination studies performed as preliminaries to long-term tests. All three species tested had the same metabolic result, a quick breakdown of the IR3535 to the carboxylic acid derivative through the liver and a rapid elimination in urine. This was seen following both iv injection and dermal application. Dermal application was not rapid or complete even after 24 hours, but little radioactivity remained in the carcass after 72 hours (with a 24 hour exposure).

The *in vitro* study on the hepatocytes was not done by GLP and did not include QA. None of the others met Guideline requirements, even though GLP and QA were provided.

Guideline §95-9: Efficacy Studies in Biting Flies, Ticks, Mosquitos, Lice (MRIDs 442708-36, 37, 38, 40) Several repellency efficacy studies on various species were performed both *in vitro* and *in vivo*. None of these studies followed GLP, or included QA. Most were range-finding studies or summaries of previously performed studies. They did demonstrate the effectiveness of IR3535 on a number of different types of pests.

Supplemental and Additional Studies: Chemistry, 4-Week Oral Toxicity in Rats, 2-Week Gavage Toxicity in Rabbits, 10-Day Investigatory Studies in Rabbits (MRIDs 442979-01, 442708-23, 24, 27, 28, 32, 39) None of these studies meet specific guidelines, but were generally used for preparatory and background information gathering.

BPB's Conclusions and Recommendations: Many of the studies discussed above are not required for the registration of IR3535 as a manufacturing-use product, as the acute toxicity studies would not have triggered the next level of testing. The information provided is adequate to approve the registration.

DATA EVALUATION REVIEW FOR ACUTE ORAL TOXICITY (§81-1)

Product Manager: 90 **Reviewer**: Carol Frazer, Ph.D.
MRID No.: 442708-20 **Report Date**: 30 January 1997
Testing Laboratory: Institute of Toxicology, Merck KGaA
Report No.: T14215
Author(s): Dr. A. Heusener
Species: HsdCpb: WU rat
Weight: males: 178-189 g; females: 164-175 g
Age: 6 to 9 weeks
Sex: 5 males, 5 females
Source: Harlan-Winkelmann GmbH, Borchen
Test Material: Insect Repellent 3535 (lot/batch numbers not included, nor physical state and appearance, although the fact density is reported leads one to believe it is a liquid)
Quality Assurance (40 CFR §160.12): Included, acceptable

Summary:

1. **LD_{50} (mg/kg)**: >5,000
2. **Toxicity Category**: IV
3. **Classification**: Acceptable

Procedure: Animals acclimated at least 7 days. Rats weighed prior to treatment and on days 2, 4, 6, 8, 11, 13 and 15. Food withheld for ~17 hours before dosing and 4 hours thereafter. Rats dosed by gavage for Limit Test at 5,000 mg/kg using a dilution in water of 250 g/l and volume of 20.0 ml/kg. Animals observed the first 6 hours after dosing, and daily thereafter for 15 days, then necropsied.

Results: No deaths observed at this dose. The LD_{50} of Insect Repellent is >5,000 mg/kg. Clinical signs in rats started at 1 to 15 min after dosing and lasted up to day 2. Clinical signs in all animals in both sexes included salivation and locomotor disturbance on the first day. Also on the first day, one male had incomplete eyelid closure, one female had abdominal posture and on the second day a different female retained feces. Weight gains normal and no abnormalities at necropsy.

BPB's Comment: No information on exactly what was tested (batch/lot number, physical state or appearance), although density reported leads to the assumption it was a liquid. Except for this, BPB finds this material meets the requirement for acute oral toxicity testing §81-1.

DATA EVALUATION REVIEW FOR ACUTE INHALATION TOXICITY (§81-3)

Product Manager: 90 **Reviewer**: Carol Frazer, Ph.D.
MRID No.: 442708-21 **Report Date**: July 8, 1996
Testing Laboratory: Department of Toxicology, BASF Aktiengesellschaft
Report No.: Proj. No. 13I0189/957012
Author(s): Dr. A.O. Gamer, Dr. E. Leibold, Dr. H.D. Hoffman
Species: Wistar rat (SPF Wistar/Chbb:THOM)
Weight: males: 258-273 g, females: 190-204 g
Age: 8-9 weeks
Sex: 5 males, 5 females
Source: Dr. K. Thomae GmbH, Biberach, FRG
Test Material: Insekt-Repellent 3535, Batch No.: K 20961687; colorless to yellowish liquid
Quality Assurance (40 CFR §160.12): Included, acceptable

Summary:
1. **LC_{50} (mg/L)**: >5.1
2. **Toxicity Category**: IV
3. **Classification**: Acceptable

Procedure: (Acclimation time for test animals not given in study report.) Test substance used as provided. Observations made several times during the 4-hour exposure, and subsequently twice on weekdays and once on weekends throughout study period. Body weights taken prior to exposure, one week later and at end of observation time (14 days). Necropsy performed on all animals.

Exposure conditions (measured continuously and recorded once): chamber: temperature (22 ± 2°C), humidity not measured, oxygen level not recorded; ambient: temperature (20-24 °C), humidity (30-70 % RH)

Exposure chamber: nose-only 5 liter glass-steel INA 20

Particle size analysis performed on a single sample of material drawn from the breathing zone of the animals and measured with a Andersen Stack Sampler Mark III at 75 minutes into exposure.

Concentrations measured analytically by gas chromatography. Samples taken hourly for four hours from the breathing zone of animals and collected on filters.

Aerosol generated by a Schlick Mod. 970 atomizer supplied by a continuous infusion pump (B. Bruan Perfusor VII) with filtered compressed air and passing through an aerosol mixing vessel and a cyclonic separator adjusted to generate appropriate aerosol.

Airflow set for test = 1500 lph, air change of about 27 times per hour calculated from this rate. T_{99} = 10 minutes.

Results: No deaths observed at the maximum achievable LC_{50} of this chemical for rats of >5.1 mg/L, with an MMAD of 1.3 micromolar and a GSD of 2.98. Escape attempts for all females and 4/5 males occurred only during the exposure period. Two animals showed substance on snouts after exposure. Other clinical signs observed included irregular respiration in all females during exposure, and 3/5 males, accelerated respiration in all test animals up to 2 days, and intermittent respiration in 1 male on the first day. Bloody nose discharge in 3 males and 2 females lasted up to the second day after exposure. Piloerection in all animals was the symptom that lasted for the greatest length of time, up to 6 days. No signs seen after day 7. All animals gained weight appropriately over the observation period. No abnormalities detected upon necropsy.

BPB's Comment: No mention of whether or not animals acclimated before testing as required by GLP, but the protocol provided specifies 1 week adaptation before testing. The concentration of test substance at the first reading (60 minutes) was relatively low (3.68 mg/l) compared with the final average (5.1). The MMAD was only measured once and that only 15 minutes after the concentration was measured at 3.68. However, even 3.68 is considered a Toxicity IV, and no further testing is needed. BPB will accept the data as presented for this study, §81-3.

DATA REVIEW FOR PRIMARY EYE IRRITATION TESTING (§81-4)

Product Manager: 90 **Reviewer:** Carol Frazer, Ph.D.
MRID No.: 442708-22 **Report Date:** April 2, 1996
Testing Laboratory: Institute of Toxicology, Merck KGaA
Report No.: T13919
Author(s): Dr. A. Heusener
Species: Iva:NZW, albino rabbit
Weight: 3.73-4.47 kg
Age: ~37-38 weeks
Sex: 3 male, 3 female
Source: SAVO-Ivanovas, Kisslegg
Test Material: Insect Repellent 3535, Batch No.: K20961687; liquid
Quality Assurance (40 CFR §160.12): Included, acceptable

Summary:

1. **Toxicity Category:** II

2. **Classification** Acceptable

Procedure: Animals acclimated at least 7 days before test. Eyes of rabbits examined 24 hours prior to treatment with an ophthalmoscope and 0.15% fluorescein instillation. One-tenth milliliter of test substance instilled into conjunctival sac of left eye and eyelids held together about 1 second. Contralateral eye served as control. Twenty-four hours after treatment, eyes were washed with physiological saline. Ocular responses were recorded at one hour, on days 1, 2, and subsequently until day 15 post-instillation. Fluorescein examination again used at end of study. All rabbits weighed immediately before treatment and on days 5, 8, 11 and 15. Abnormal pharmacologic or toxic signs noted. Draize scoring system used and presented in study report.

Results: This test substance causes substantial eye injury. Corneal opacity, conjunctivitis, chemosis and discharge observed in all test animals. Corneal effects were most significant with one animal yielding grade 1 opacity until day 7. No iritis seen in any test subject. All 6 rabbits had grade 3 discharge at the first reading, which decreased rapidly until non-significant by 48 hours. Chemosis and hyperemia progressed no higher than grade 2 by 24 hours, again reduced quickly, and were non-significant by the 6th day.

BPB's Comment: BPB accepts the above study as completing the requirements for §81-4.

DATA EVALUATION REVIEW FOR DERMAL SENSITIZATION (§81-6)

Product Manager: 90 **Reviewer**: Carol Frazer, Ph.D.
MRID No.: 442979-02 **Report Date**: March 13, 1997
Testing Laboratory: Hill Top Research Ltd.
Report No.: 96-8304-21
Author(s): Teresa D. Morris, B.S.
Species: Hartley albino guinea pig
Weight: 348 - 482 g
Age: ~8 weeks
Sex: 43 animals, ~ equal amounts of each sex
Source: Harlan Sprague Dawley, Inc., Indianapolis, IN
Test Material: IR3535 Technical, Batch Number: K20961687; clear, colorless liquid
Positive Control: α-Hexylcinnamaldehyde: induction - 2.5% in 95% ethanol; challenge - 5% and 2.5% w/v concentration in acetone
Quality Assurance (40 CFR §160.12): Included, acceptable

Method: Modified Buehler

Summary: 1. **Rating**: Non-sensitizer

2. **Classification**: Acceptable

Procedure: Acclimation period at least 5 days. Irritation screening undiluted, 50%, 25% and 10% w/v in ethanol and acetone. Both induction and challenge concentrations were 100%. During induction, 20 animals treated with 0.3 ml test substance once each week for 3 weeks on pre-clipped test sites under an occlusive 25 mm Hill Top Chamber secured with rubber dental dam pulled taut and fastened to the bottom of a restrainer with clips. After 6 hours of exposure, dental dam unfastened, restrainers and chambers removed. Two weeks after last induction exposure, test and positive control subjects, along with 10 naive control animals were treated with appropriate substance in the same manner. The day following irritation screening and primary challenge exposure, all animals treated with a commercial depilatory which was left on for no longer than 15 minutes. The depilatory was thoroughly removed. Skin scores taken at 24 and 48 hours following screening and challenge. Body weights taken on days 0 and at completion of study. Report included scoring information.

Results: Screening results at 24 and 48 hours at all dose levels were primarily ± in either ethanol or acetone. No significant dermal sensitization exhibited with this test substance. All test animals and naive controls exhibited the lowest reading after challenge with test material at 24 hours, and this reduced to zero in approximately 50% of the animals at 48 hours. All animals gained weight with no toxic reactions. Nearly 100% of the positive controls had grade 1 reading at both 24 and 48 hours, at both 5% and 2.5%.

BPB's Comment: This meets §81-6 requirements and indicates test material a non-sensitizer.

CONSUMER INFORMATION REQUESTED AND RECEIVED FROM EUROPEAN PRODUCERS CONCERNING THE USE OF IR3535™ IN FORMULATIONS (§152-16)

Product Manager:	90	**Reviewer**:	Carol Frazer, Ph.D.
MRID No.:	442979-08	**Report Date**:	Not applicable
Compiler:	Merck KGaA		
Report No.:	Not applicable		
Author(s):	S.D. Phillips, M.S.		

Dr. Jalalian of Merck KGaA requested all consumers of IR3535 for use data and any toxicologic complaints pertaining to the product. This was required before Merck could register it in the USA. Thirteen companies responded, and 12 stated they hadn't received any complaints. One respondent, Biomed Ag, Dubendorf/Switzerland, reported that after 5 years use, alone or in combination with other active ingredients there had been reported "one number of skin irritancy or adverse reactions (sic)."

DATA EVALUATION REVIEW FOR SUBCHRONIC DERMAL TOXICITY (§82-3)

Product Manager: 90 **Reviewer**: Carol Frazer, Ph.D.
MRID No.: 442979-03 **Report Date**: 5 Aug 1996
Testing Laboratory: RCC, Research & Consulting Company, Ltd.; BRL, Biological Research Laboratories, Ltd.
Report No.: 398834
Author(s): Dr. Th. Pfister, H. Luetkemeier, Dr. D. Stegehuis, Dr. K. Weber
Species: HanIbm: WIST (SPF) rat
Weight: males: 185-226 g; females: 285-214 g
Age: males: 8 weeks; females: 10 weeks
Sex: 50 males, 50 females
Source: BRL, Biological Research Laboratories, Ltd., Füllinsdorf, Switzerland
Test Material: Insect Repellent 3535 (Art.No. 111887), Batch Number K 20961686; faintly yellowish liquid
Quality Assurance (40 CFR §160.12): Included, acceptable

Summary: 1. **NOEL (mg/kg/day)**: 3,000

2. **Classification**: Acceptable

Procedure: Animals acclimated 1 week after health examination. Test animals clipped on approximately 10% of the dorsum and re-shaved several times throughout the study. Four dose levels used for this study: 0 (in Leercreme 10/L), 100 (2% in Leercreme 10-01/L), 1,000 (20% in Leercreme 10-02/L) and 3,000 (60% in Leercreme 10-03/L) mg/kg/day using Leercreme as the vehicle. The use of different vehicles was necessary to achieve similar consistency in the creams at all concentrations to assure the accuracy of the application procedure. INSECT REPELLENT 3535 was mixed with vehicles; homogeneity ensured by stirring the test article dilutions for several minutes before and, if appropriate, at several occasions during the daily dosing procedure.

Test substance spread over the shaved area as uniformly as possible and covered with an occlusive dressing (patch of filter paper covered with a strip of aluminum) wrapped around the abdomen and fixed with an elastic adhesive bandage for 6 hours/day, 7 days/week for at least 90 treatments. One group of 10 animals/sex at the high dose was maintained after dosing was completed for an additional period of 4 weeks. Fresh dressing material used for each daily application. After each 6 hour exposure period, dressing removed carefully, excess test article gently washed off with lukewarm tap water, and skin dried with a paper towel.

All animals observed at least twice daily for mortality/viability and once daily for systemic reactions. Skin reactions, erythema and edema, were recorded immediately prior to first application and before each new application daily thereafter throughout the treatment. Evaluation scale given in study report. Body weights recorded weekly during the study, and at necropsy. Ophthalmoscopic examinations on both eyes of all animals performed before dosing,

toward the end of the dosing period, and toward the end of the recovery period on all surviving animals. Between 10 and 90 minutes after instillation of 2 drops of a mydriatic agent, the cornea, lens, anterior chamber, vitreous body and fundus were examined, under dimmed light, using an ophthalmoscope.

Following an 18-hour fast, blood samples for hematology and clinical biochemistry collected from all animals to be sacrificed after 13 weeks, and from recovering animals after 17 weeks. Urinalyses for all animals performed on urine collected from subjects during the fasting period.

All animals weighed and necropsied at end of observation period, or when animal died, and descriptions of all macroscopic abnormalities recorded. Prior to sacrifice, animals fasted for approximately 18 hours with free access to water. Organ weights taken and samples of chosen organs fixed and retained.

Results: No evidence of systemic toxicity following this 90-day study. The NOAEL of IR3535 in rats after dermal administration for 90 days was 3,000 mg/kg/day. Two animals in the high-dose group (3000 mg/kg/day) died spontaneously before scheduled necropsy. One female died on day 81 without systemic clinical signs. Spontaneous liver lobe torsion observed in this animal at necropsy considered to have been the cause of death. A male assigned to the recovery group was found dead on test day 113. This animal showed slight sedation, dyspnea, ruffled fur and emaciation before death. Cause of death could not be determined.

One high-dose female showed moderate alopecia of legs and abdomen and slight emaciation towards the end of the recovery period; another female of this group showed transient slight swelling and alopecia around the right eye which had been injured during the bleeding procedure at the end of the treatment period.

No other systemic clinical signs observed in any other animals.

Local clinical signs of scaling and focal erythema, generally very slight to moderate were frequently noted at the treatment site. Edema formation did not occur. The severity of lesions generally appeared constant over the various groups for both males and females. Changes of this nature and severity are common findings in laboratory rodents after repeated shaving and bandaging. In most of the animals assigned for recovery, local signs disappeared during the treatment period or were still present at the end of the recovery period. Minor grades of inflammatory changes (inflammatory cell foci fibroplasia and epidermal inflammation) seen at necropsy in a few animals from each group. Overall findings considered to be indicators of minimal irritant effects in all animals due to shaving and bandaging.

There were transient or non-dose related effects in each group, but no significant changes in absolute or relative food consumption of treated animals. Nor was there any effect on body weight gain. At necropsy, there were no changes in absolute or relative organ weights attributable to the test article.

Ophthalmoscopic findings noted at random in every dose group. They included persistent pupillary membrane, persistent hyaloid vessels, corneal opacity, lenticular opacity posterior and anterior synechia. Again, these findings are similar to those known to occur spontaneously in rats of this age and strain, and were present at similar incidences in control and treated groups.

There were no effects of toxicologic significance on hematology, clinical biochemistry and urinalysis after 13 weeks of treatment nor at the end of the recovery period. All data within historical levels, except for a lower urine pH in males of the high-dose group after 13 weeks of treatment.

BPB's Comment: This study meets the requirements of §82-3 and indicates the test material is not a subchronic dermal toxicant in the rat.

Developmental Toxicity

Merck submitted five studies related to the developmental toxicity of **Insect Repellent 3535**: two complete rabbit developmental studies on the Himalayan and New Zealand White strain (DERs follow) and a range-finding study on the New Zealand White (DERs given), and two supplemental investigatory studies on the Himalayan and New Zealand White rabbit strains.

The first study (MRID 442708-26), with the protocol dated March 31, 1995, and final report dated July 22, 1996, used rabbits of the Himalayan strain and the study was performed by the Institute of Toxicology at Merck KGaA in the Federal Republic of Germany in accordance with the German, OECD and EPA Good Laboratory Practice standards. The laboratory flagged the study as equivocal and inappropriate for use for hazard evaluation purposes as a result of 40 CFR 158.34, criteria number 5. Doses used per group of 15 were 1 ml/kg (undiluted), 0.3 ml/kg, 0.1 ml/kg, and vehicle control (demineralized water) on days 6 - 19. As the **Insect Repellent 3535** is not water-soluble at the doses used, a direct dose was gavaged. At least 13 of 15 animals in each dose group became pregnant.

One rabbit in the high dose group aborted and was later killed in extremis. A further five high-dose rabbits and one mid-dose animal died or were killed for humane reasons. Except for a gavaging accident involving one rabbit in the high-dose group, the pathologic findings did not permit any conclusion as to cause of death. Food consumption was dose-dependently reduced from days 6 through 10 in all dose-groups and from days 10-14 in the high-dose group. Water consumption in the high-dose group was also significantly reduced on the first 2 days of treatment. This is definitely treatment-related, as is the weight-loss in the high-dose group. However, final carcass weights in all dose groups were not significantly different from the control.

The number of corpora lutea and implantations were essentially similar in all groups. A trend toward higher resorption rates was seen in the low-dose group, while clearly increased resorption rates were seen in the higher groups. Thus, due to the higher post-implantation losses, the number of live fetuses in the mid- and high-dose groups was reduced. No dead fetuses were found in any group, and survival rates in the viability test was not impaired by treatment of the dam. Fetal weights were similar in all groups. The occurrence of congenital runts was confined to one fetus in the mid-dose group. Sex distribution similar in all groups.

No NOAEL identified for maternal toxicity. Teratogenic findings diagnosed in the low- and mid-dose groups were not dose-dependent; findings and incidence corresponded to spontaneous findings observed in this strain of rabbit. Final results with no identifiable dose-related teratogenicity in the study at maternally lethal doses, and no embryolethality at the LOAEL for maternal toxicity, will require the study be repeated.

A pilot developmental study in the Himalayan rabbit at 1.5 g/kg/days 6 - 18 (Leuschner 1975a; not submitted) had indicated no apparent embryotoxic effects or malformations. A

similar study in the rat at 1.8 g/kg/days 6 - 18 (Leuschner 1975b; not submitted) also was unremarkable.

Merck KGaA also submitted two supplemental investigative studies on IR3535 with oral administration to different strains of rabbits: effects on body temperature and histopathology in the non-pregnant Himalayan rabbit (MRID 442708-27), and plasma half-life and toxicity of IR3535 on non-pregnant rabbits of Himalayan and New Zealand White strains (MRID 442708-28).

On November 15, 1995, the Study Director of the Institute of Toxicology of Merck KGaA signed a protocol to evaluate whether Insect Repellent 3535 (IR3535) affected body temperature, laboratory values and histopathology of non-pregnant female Himalayan strain rabbits. Final report signed March 11, 1997 (MRID 442708-27). Because earlier studies showed decreases in body temperatures following a dose of 1 g/kg/day in both pregnant and non-pregnant Himalayan rabbits (Gleich, 1997; not submitted), lower doses were evaluated. In two groups of five Himalayan rabbits each, at doses of 0.1 g/kg/day and 0.3 g/kg/day for 10 days, no mortality was seen. No change in food consumption. Bodyweight gain appeared normal except for brief loss of weight at dosing -- no alterations in hematologic readings, no significant clinical signs observed or reduction in body temperature noted, and only minor changes in blood chemistry. Pathologically, 2/5 rabbits in each dose group developed gastro-mucous membrane hemorrhages. Historical rate of spontaneous activity not known, but acidification of drinking water in the laboratory may have contributed to this result.

After the Himalayan strain study proved unusable, Merck immediately initiated further testing on another rabbit strain, New Zealand White. A range-finding study was initiated at WIL laboratory April 22, 1996 and signed off March 7, 1997. At doses of 50, 100, 300, 600 and 1,000 mg/kg/day and a vehicle control (1% carboxymethylcellulose) no test article related deaths or abortions occurred at any level. No external malformations or developmental variations observed in fetuses in this study.

The definitive study on New Zealand White was initiated August 20, 1996, and completed February 19, 1997. Three doses (100, 300 and 600 mg/kg/day) and a vehicle control (1% carboxymethylcellulose(w/v)/0.1% Tween® 80(w/v)) were given from days 7 through 19 of gestation to 20 females/group.

NOAEL >300 mg/kg/day for maternal toxicity and >600 mg/kg/day for developmental toxicity. No test article-related deaths or abortions occurred during this study. A higher incidence of decreased defecation in the 600 mg/kg/day group occurred during treatment was only sign attributable to IR3535. Body weight gain and food consumption inhibited in the high dose group during the first 3 days of dosing, and food consumption in this group continued slightly reduced throughout the remainder of the period. Body weight gain and food consumption were unaffected by treatment in the other dosage groups. Intrauterine growth and survival were unaffected by test article administration at any dose level. No treatment-related malformations or developmental abnormalities observed in fetuses.

A further investigative study was performed by Merck KGaA (MRID 442708-28) with a protocol signature on January 10, 1997 and final report signed on March 11, 1997. Because of the difference in results between two developmental toxicity studies performed in the Himalayan and New Zealand White rabbit strains, this investigatory study concentrated on a comparison in plasma levels between the two rabbit strains. Three female rabbits of each strain received 0.6 g/kg/day Insect Repellent 3535 for 10 days. Peak levels in the blood were between ½ hour and 1 hour following administration on day 1 and 10. The chemical was cleared from the blood within 24 hours (checked after days 1 and 10), and no accumulation noted. No difference observed between plasma half-lives. No mortality, and body weight gain appeared normal after initial weight loss. Same effect observed in other investigatory study. Histopathologic examination of the rabbits revealed atrophy of mucous membranes in 2 Himalayan and 1 New Zealand White animal, as noted in previous study.

The two investigatory studies (MRIDs 442708-27, -28) appeared to have been conducted adequately and substantiate the conclusions by the authors, that: 1) Insect Repellent 3535 did not cause a decrease in body temperature in the non-pregnant Himalayan rabbit at lower doses, and, 2) plasma levels of the chemical in the two strains evaluated were not different in the non-pregnant animals at the higher dose tested. However, neither investigatory study contraindicated the use of Himalayan rabbits for the developmental toxicity study.

Conclusions

FIFRA guidelines require two species be tested for developmental toxicity. This was not done. Because IR3535 is not significantly acutely toxic, BPB would not normally require developmental toxicity testing unless mandated by Food Quality Protection Act (FQPA). If that were the case, another species would have to be tested.

The Himalayan rabbit is not frequently used for developmental toxicity studies because of the non-consistency of its response. A simple review of information provided by the sponsor on preliminary studies as well as the final and investigatory studies indicate this to be true. The sponsor repeated the study with another strain of rabbit, the New Zealand White, and this proved acceptable. However, BPB will keep in mind the Himalayan strain's results in doing a complete evaluation of the developmental toxicity of **IR3535**.

DATA EVALUATION REVIEW OF DEVELOPMENTAL TOXICITY STUDY
WITH ORAL ADMINISTRATION TO RABBITS (§83-3)

Product Manager: 90 **Reviewer:** Carol Frazer, Ph.D.
MRID No.: 442708-26 **Report Date:** July 22, 1996
Testing Laboratory: Institute of Toxicology, Merck KGaA
Report No.: T 9382
Author(s): J. Gleich
Species: Himalayan Chbb:HM (SPF) virginal female rabbits
Weight: 2.58 - 3.40 kg
Age: 374 - 424 days
Sex: 60 females, 1 control and 3 test groups
Source: Not given
Test Material: Insect Repellent 3535, Batch Number K 20961687; faintly yellowish liquid
Quality Assurance (40 CFR §160.12): Included, acceptable

Summary: 1. **NOAEL(developmental toxicity)(mg/kg/day):** >300
LOAEL (maternal toxicity)(mg/kg/day): 100

2. **Classification:** Unacceptable

Procedure: Animals acclimated at least 3 months before study. Doses were 1 g/kg (undiluted test substance), 0.3 g/kg, 0.1 g/kg (dilutions in demineralized water) and a vehicle control group. Standard volume of 1 ml/kg gavaged followed by a 5 ml demineralized water drench. Rabbits weighed on day 0 (mating) and daily from day 6 through sacrifice. Food consumption determined twice a week and water consumption every two days. Clinical examinations performed every day before and after dosing.

All animals necropsied. Laboratory determined numbers of corpora lutea, live and dead fetuses and resorptions, both early and late. Uteri with no macroscopic evidence of implantation subsequently measured for early implantation loss. All live fetuses were kept in an incubator for 24 hours to determine their viability. Fetuses subsequently necropsied. Sex was determined by inspection of gonads. All fetuses necropsied and checked for macroscopic anomalies, and subsequently fixed and examined for skeletal abnormalities and malformations.

Results: Acceptable levels of pregnancies observed in each group (13-15). One rabbit in high-dose group aborted and later killed in extremis. A further five high-dose rabbits and one mid-dose rabbit died or were sacrificed. Except for one rabbit in the high-dose group with a gavaging accident, pathological findings did not permit any conclusions on cause of death.

Food consumption in all groups was dose-dependently reduced during the treatment period. Water consumption in the high-dose group reduced during the first 2 days of treatment. Rabbits in control, low- and mid-dose groups lost some body weight after the first treatments. Weight

losses in treatment groups became more pronounced with increasing doses and were considered treatment-related. Numbers of corpora lutea and implantations similar in all groups. A trend toward a higher resorption rate observed in the low-dose group, while clearly increased resorption rates were seen at higher doses. Thus, due to the higher post-implantation losses, number of live fetuses in the higher dose groups reduced. No dead fetuses found in any group, and survival of fetuses not impaired. Fetal weight and sex distribution similar in all groups. One fetus demonstrated terata, but this was within the historical control levels.

No embryolethality seen at any dose, but maternal toxicity seen at all doses, therefore, no NOAEL can be determined.

BPB's Comment: This is not an appropriate developmental toxicity study (§83-3) for the Himalayan rabbit as a maternal NOAEL was not achieved. The information will, however, be utilized in completing a definitive evaluation for the developmental toxicity of this chemical.

DATA EVALUATION REVIEW OF A TWO-WEEK REPEATED DOSE TOXICITY STUDY OF IR3535 IN NON-PREGNANT RABBITS

Product Manager:	90	**Reviewer:**	Carol Frazer, Ph.D.
MRID No.:	442708-39	**Report Date:**	January 6, 1997
Testing Laboratory:	WIL Research Laboratories, Inc.		
Report No.:	WIL-149022		
Author(s):	James L. Schardein, M.S., A.T.S.		
Species:	New Zealand White rabbit		
Weight:	3234 - 4519 g		
Age:	19 - 34 weeks		
Sex:	16 females		
Source:	Hazleton Research Products, Inc., Denver, Pennsylvania		
Test Material:	Insect Repellent 3535, Batch: K20961687; clear colorless liquid		
Quality Assurance (40 CFR §160.12):	Included, acceptable		

Summary: **LOAEL(mg/kg):** >600 mg/kg/day

Procedure: A vehicle (1% carboxymethylcellulose and 0.1% Tween™ 80) control and one treatment (600 mg/kg/day) dose group (6/group) utilized in this 13-day gavage oral study. Dosage volume of 5.0 ml/kg used for both groups. All animals observed daily for overt signs of toxicity, body weights, food consumption and body temperatures. Necropsy with macroscopic examination performed on all rabbits.

Results: All animals survived to scheduled sacrifice. No clinical signs observed and body temperatures unaffected by treatment. Food consumption was however, slightly reduced during the first few days of treatment and subsequent weight gain diminished. No test article administration-related findings noted at necropsy.

BPB's Comment: This study in the female rabbit indicates the test material is not severely toxic at this oral dose and, the dose suitable for a complete developmental toxicity study. The study also highlighted the method of administration, a 12-gauge stainless steel gavage cannula, also considered acceptable.

DATA EVALUATION REVIEW OF A TWO WEEK REPEATED DOSE TOXICITY STUDY OF IR3535 IN NON-PREGNANT RABBITS (§83-3)

Product Manager: 90 **Reviewer:** Carol Frazer, Ph.D.
MRID No.: 442708-39 **Report Date:** January 6, 1997
Testing Laboratory: WIL Research Laboratories, Inc.
Report No.: WIL-149022
Author(s): James L. Schardein, M.S., A.T.S.
Species: New Zealand White female rabbits
Weight: 3234-4519 g
Age: 19-34 weeks
Sex: 12 females
Source: Hazleton Research Products, Inc., Denver, Pennsylvania
Test Material: Insect Repellent 3535, Batch Number K 20961687; clear, colorless liquid
Quality Assurance (40 CFR §160.12): Included, acceptable

Summary: **NOEL(mg/kg/day):** >600

Procedure: Animals acclimated 4 weeks. Animals (6F/group) dosed at 600 mg/kg/day at 120 mg/ml, and with a vehicle control group (1% carboxymethylcellulose(w/v)/0.1% Tween® 80 (w/v)) treated for two weeks. Doses administered in a volume of 5 ml/kg via a rubber catheter by gavage.

Animals observed twice daily for mortality and morbidity, with a detailed physical examination recorded prior to dose administration. Animals observed for toxicity signs one hour after dosing. Body weights and food consumption recorded daily. Body temperatures recorded daily, approximately two hours post-dosing. All rabbits necropsied.

Results: No deaths occurred during this study, nor were any clinical signs noted that seemed test article related.

Slight mean body weight losses were noted in treatment group on study days 1, 2 and 4. Mean body weights in this group slightly lower than the control group on study days 1 through 5, but no other trends noted throughout the remainder of the study. Food consumption slightly inhibited by test article administration the first five days of study, but not the rest of the study. Body temperatures in treated group were comparable to control group throughout the study.

One treated animal had a cystic oviduct, one had multiple dark red areas in the stomach and an accessory spleen and abscesses in the thoracic cavity. In the control group, one had agenesis of the right kidney and ureter, an enlarged left kidney and an accessory spleen. Another control rabbit also had an accessory spleen. Nothing seemed treatment-related.

BPB's Comment: This study is an acceptable preliminary supplement for a developmental toxicity study (§83-3) in the New Zealand White rabbit.

DATA EVALUATION REVIEW OF DOSE-RANGE FINDING
DEVELOPMENTAL TOXICITY STUDY OF IR3535 IN RABBITS (§83-3)

Product Manager: 90 **Reviewer:** Carol Frazer, Ph.D.
MRID No.: 442708-25 **Report Date:** March 7, 1997
Testing Laboratory: WIL Research Laboratories, Inc.
Report No.: WIL-149020
Author(s): James L. Schardein, M.S., A.T.S.
Species: New Zealand White [Hra:(NZW)SPF] virgin female rabbits
Weight: 2954 - 3745 g at reception; 3225 - 4164 g at insemination
Age: 5½ months old at reception; 6½ months old at insemination
Sex: 42 females, 1 control and 5 test groups
Source: Hazleton Research Products, Inc., Denver, Pennsylvania
Test Material: Insect Repellent 3535, Batch Number K 20961687; clear, colorless liquid
Quality Assurance (40 CFR §160.12): Included, acceptable

Summary: 1. **NOAEL(maternal toxicity)(mg/kg/day):** >300
NOAEL(fetal toxicity)(mg/kg/day): >1,000

2. **Classification:** Acceptable

Procedure: Animals acclimated 3 weeks before artificial insemination. Test substance doses were 50, 100, 300, 600 and 1,000 mg/kg, and a vehicle control group (1% carboxymethylcellulose(w/v)/0.1% Tween® 80(w/v)) given to 6 females/group from days 7 through 19 of gestation. Doses administered in a volume of 5 ml/kg via a rubber catheter by gavage.

Animals inseminated on day 0. Rabbits observed daily in morning and afternoon for morbidity and mortality through day 29 (end of treatment). Toxicity signs checked one hour after dosing. Detailed clinical observations on all animals maintained throughout study. Animals weighed on days 0, 7-20 (daily), and on days 24 and 29. Gravid uterine weight collected and net body weight calculated at scheduled laparohysterectomy. Daily food consumption measured.

On day 29, all surviving females euthanized, uterus and ovaries excised, and number of corpora lutea on each ovary recorded. Trimmed uterus weighed, opened. Number and location of all fetuses, early and late resorptions, and total number of implantation sites recorded. Uteri with no macroscopic evidence of implantation subsequently measured for early implantation loss.

Each fetus weighed and tagged for identification. A detailed external examination of each fetus conducted including an examination of eyes, palate and external orifices. Crown-rump measurements recorded for late resorptions (if present) and tissue discarded. All does necropsied.

Results: No test article-related deaths or abortions occurred during study. Six does (2 in the lowest dose group, 1 each in control, 100, 600 and 1,000 groups) died during the study, and these were considered administration-related, rather than test article-related. One female in the 1,000 mg/kg/day group was euthanized because of mechanical trauma (fractured vertebrae) and an animal in the 600 mg/kg/day group was euthanized on gestation day 18. This female was prostrate and had labored respiration, mitosis drooping eyelids, eyes that appeared dark and decreased defecation on the day of euthanization. One doe in the 50 mg/kg/day group aborted on gestation day 23. This female also had indications of intubation error (brown granular contents observed in trachea) upon necropsy.

Clinical findings in treated groups, such as clear nasal discharge, soft stool and decreased defecation, occurred in single animals per group or were considered non dose-related. A statistically significant mean body weight loss was observed in the 600 mg/kg/day group during gestation days 7-10, but, over the period 10-13, it was comparable to control. Mean body weight losses occurred during the period of 13-20 and 7-20, but these differences from the control group were insignificant. The higher dose group showed no statistically significant differences on any point. Neither were there adverse effects in the lower dose groups. Food consumption, however, was inhibited in the two high dose groups during the first 3 days of dosing, and it continued slightly reduced throughout the remainder of the treatment period. During the post-treatment period, food consumption increased to control levels. Body weight gain and food consumption were unaffected by treatment in the other dosage groups. Mean gravid uterine weights, net body weights and net body weight changes were unaffected by test article administration at any dose level. A mean net body weight loss occurred at the 50 mg/kg/day group, but mean net body weight gains were noted at all higher dose levels, and this was not considered to be treatment-related. No treatment-related malformations or developmental abnormalities observed in fetuses.

At necropsy, no internal findings related to test article administration were observed at any dose level. Intrauterine growth and survival were unaffected by treatment at any dose. No external malformations or developmental variations observed in fetuses in this study.

BPB's Comment: This study is an appropriate dose range-finding study for a definitive developmental toxicity study (§83-3) in the New Zealand White rabbit.

DATA EVALUATION REVIEW OF A DEVELOPMENTAL TOXICITY STUDY OF IR3535 IN RABBITS (§83-3)

Product Manager: 90 **Reviewer:** Carol Frazer, Ph.D.
MRID No.: 443233-01 **Report Date:** February 19, 1997
Testing Laboratory: WIL Research Laboratories, Inc.
Report No.: WIL-149021
Author(s): James L. Schardein, M.S., A.T.S.
Species: New Zealand White [Hra:(NZW)SPF] virgin female rabbits
Weight: 2178 - 3066 g at reception,
Age: 4½ months old at reception, 7 months old at insemination
Sex: 60 females, 1 control and 3 test groups
Source: Hazleton Research Products, Inc., Denver, Pennsylvania
Test Material: Insect Repellent 3535, Batch Number K 20961687; clear, colorless liquid
Quality Assurance (40 CFR §160.12): Included, acceptable

Summary: 1. **NOAEL(maternal toxicity)(mg/kg/day):** >300
NOAEL(developmental toxicity)(mg/kg/day): >600

2. **Classification:** Acceptable

Procedure: Animals acclimated for 9 weeks before artificial insemination. Test substance doses were 100, 300 and 600 mg/kg, and a vehicle control group (1% carboxymethylcellulose(w/v)/0.1% Tween® 80(w/v)) given to 20 females/group from days 7 through 19 of gestation. Doses were administered in a volume of 5 ml/kg via a cannula by gavage.

Animals inseminated on day 0. Rabbits observed for morbidity and mortality daily in morning and afternoon through day 29 (end of treatment). Toxicity signs checked one hour after dosing. Detailed clinical observations on all animals maintained throughout study. Animals weighed on days 0, 7-20 (daily), and on days 24 and 29. Gravid uterine weights collected and net body weight calculated at the scheduled laparohysterectomy. Daily food consumption measured.

On day 29, all surviving females euthanized, uterus and ovaries excised and number of corpora lutea on each ovary recorded. Trimmed uteri weighed, then opened. Numbers and locations of all fetuses, early and late resorptions and total number of implantation sites recorded. Uteri with no macroscopic evidence of implantation subsequently measured for early implantation loss.

Each fetus weighed and tagged for identification. A detailed external examination of each fetus was conducted including an examination of the eyes, palate and external orifices. Crown-rump measurements recorded for late resorptions and tissue discarded. Sex of each fetus

determined internally and each fetus examined viscerally. Brain of each fetus examined by mid-coronal slice. All carcasses eviscerated and fixed for skeletal examination.

Results: No test article-related deaths or abortions occurred during this study. A higher incidence of decreased defecation in the 600 mg/kg/day group occurring during treatment only sign attributable to IR3535. Body weight gain and food consumption inhibited in the high dose group during the first 3 days of dosing; food consumption in this group continued slightly reduced throughout remainder of period. Body weight gain and food consumption unaffected by treatment in other dosage groups. Intrauterine growth and survival were unaffected by test article administration at any dose level. No treatment-related malformations or developmental abnormalities observed in fetuses.

One 300 mg/kg/day animal died on day 8, apparently from a dosing error. One female each in the control and 600 mg/kg/day groups aborted on gestation day 25. For all these rabbits, a gross necropsy was performed on day of death or abortion. Maternal tissues were preserved for possible future study. In addition, number and location of implantation sites and corpora lutea and viable fetuses were recorded. Recognizable fetuses examined externally and preserved.

BPB's Comment: This study meets the requirements of §83-3 and indicates the test material is not a developmental toxicant in the New Zealand White rabbit.

DATA EVALUATION REVIEW OF 2-GENERATION STUDY
WITH ORAL ADMINISTRATION TO RATS (§83-4)

Product Manager: 90 **Reviewer:** Carol Frazer, Ph.D.
MRID No.: 442979-04 **Report Date:** February 7, 1997
Testing Laboratory: Institute of Toxicology, Merck KGaA
Report No.: T 9381
Author(s): J. Gleich
Species: Wistar HSD/WIN:Wu rat
Weight: 2178 - 3066 g at reception,
Age: 8 weeks
Sex: 100 males, 100 females: 1 control and 3 test groups
Source: Harlan Winkelmann, Versuchstierzucht, in 33178 Borchen/Paderborn
Test Material: Insect Repellent 3535, Batch Number K 20961687; faintly yellowish liquid; specific gravity of substance 0.999, therefore 1 ml ~ 1 g
Vehicle: 1% aqueous Methocel®-Tween 80® solution, composed of 0.5% Methocel® + 0.5% Tween 80®, during the first 3 weeks, demineralized water after week 3
Quality Assurance (40 CFR §160.12): Included, acceptable

Summary: 1. **NOAEL (ml/kg/day):** 0.3
LOAEL (ml/kg/day): 1.0

2. **Classification:** Acceptable

Procedure: Animals acclimated for 7 days. Doses given by gavage were control, 0.1 ml/kg, 0.3 ml/kg and 1.0 ml/kg, in a volume of 5.0 ml. Rats were treated for 10 weeks before mating, and during the mating period until killed (males after the mating period, females after lactation period). Weanlings (25/sex) from each group selected from the P_0 generation on postnatal day 28 and reared to maturity. Remaining pups killed. Tests for physical and functional development performed during the lactation period, and special behavioral tests performed after weaning. F_1 generation treated 9-11 weeks before and during the mating period. F_2 pups reared until postnatal day 21 and killed.

All animals examined for mortality and morbidity twice a day during mating and growth periods, and detailed clinical examinations conducted once a week during growth and breeding periods. Body weights taken at beginning of study and weekly through growth and mating periods: daily for maternal animals during gestation and lactation periods; weekly for paternal animals post-mating until sacrifice. For all animals, food consumption measured weekly and water consumption measured every 3 days during pre-mating period.

One male caged with one female during mating for 2 weeks. Vaginal smears taken daily during mating period. If animals observed to mate twice, day 0 of gestation timed from 2nd mating. If no sperm found after 14 days observation, no further matings tried. In the high dose

of F_1 generation, 2 more males than females died. For this reason, 2 males assigned to 2 females each. Matings of siblings avoided. Mated females placed individually in cages through gestation and lactation. During pregnancy, animals observed daily on the hour between 6:30 am and 4:00 pm from day 20 through delivery. Animals littering between 6:30 am and 4:00 pm observed during birth for behavioral disturbances, and all dams daily throughout lactation for impairment of rearing and nursing instinct.

Pup observations included time of birth and daily watches for mortality, weight at birth, and sex of each pup. These were checked further on days 4, 7, 21 and 28 for the F_1 generation and up to day 21 for the F_2 generation. External alterations of pups checked daily, including 100% success attainment of unfolding of pinnae from day 1, eruption of incisors from day 9, opening of external auditory ductus from day 10, opening of eyelids from day 12, and air righting from day 15. Developmental status of fur observed starting on day 10, and papillary light reflex, pain sensitivity and auditory startle reflexes on day 21. Additional observations for 100% success made on F_1 generation included vaginal opening from day 28, balanopreputial skinfold from day 45, rotarod test during weeks 5 to 7, shock avoidance during weeks 7 to 10 and activity measurement during weeks 9 to 14.

All surviving parental males sacrificed as soon as possible after the mating period. Maternal animals sacrificed after the litter in each generation weaned. Non-mated, non-pregnant females and those with only implantations were also sacrificed and necropsied. Maternal animals whose pups died were sacrificed earlier. Spermatozoa were collected from each male, and motility, morphology and number of sperm calculated. Stillborn pups and those dying during lactation, were externally then internally examined and, after staining, examined for malformations unless cannibalized. All F_1 pups except those selected for rearing and entire F_2 generation necropsied.

Results: In the high-dose group of the parental generation, one male convulsed and died and one female was found dead with no previous symptoms. A low-dose female also showed convulsions on one day only but recovered. In the high-dose group of the F_1 generation, one male and one female died as a result of administration error, and a further two males and one female were found dead with no previous symptoms. Additionally, two F_1 generation low-dose males died under anaesthesia for blood removal.

There were no apparent treatment-related clinical observations apart from a resistance to dosing. In the high-dose group, in both sexes, this manifested as struggling during dose administration, rooting in the sawdust after dosing and, for the F_1 generation only, excessive grooming. In the low-dose group, the only signs were excessive grooming seen for parental females and both sexes of the F_1 generation. These findings are considered to be related to taste aversion and not toxicity.

No consistent effects of treatment on body weights, food or water consumption of parents in either generation. There was a dose-related increase in liver and kidney weights in the parents in the mid- and high-dose groups. The difference was statistically significant for both organs of the parental males, for kidneys of the F_1 generation males and for livers of the F_1 generation females.

The kidneys of the F_1 generation females were also significantly increased, but for the high-dose only. Adrenal weights of high-dose males in both generations were significantly increased as was the spleen weight of the high-dose females in the parental generation only. There were, however, no histopathologic findings or morphometric changes to correlate with the increased weights.

There were also no effects of treatment on sperm parameters, estrous cycles, mating or fertility. No effects on duration of pregnancy or numbers of litters and implantations. In the first generation litters, there was a higher incidence of still-born pups and pup deaths in the immediate neonatal period in the mid- and high-dose groups compared with the control, but this was not repeated in the second generation litters. Growth and development of pups was unaffected in both generations.

BPB's Comment: This study meets the requirements of §83-4 and indicates the test material is not a reproductive toxicant in the Wistar rat.

DATA EVALUATION REVIEW FOR BACTERIAL MUTAGENICITY ASSAY, SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI (§84-2)

Product Manager:	90	**Reviewer:**	Carol Frazer, Ph.D.
MRID No.:	442708-29	**Report Date:**	July 31, 1996
Testing Laboratory:	Institute of Toxicology, Merck KGaA		
Report No.:	40/53/96		
Author(s):	D. Utesch		
Test Material:	Insect Repellent 3535, Batch No. K20961687; clear colorless liquid		
Cell Source:	Dr. Bruce Ames, University of California at Berkeley, California		
Solvent Used:	Ethanol (1%)		
Control Materials:	Negative: solvent Positive: Nonactivation -- daunomycin, N-ethyl-N'-nitro-N-nitrodoguanidine, 9-aminoacridine, cumene hydroperoxide; Activation -- 2-aminoanthracene, benzo[a]pyrene		
Activation:	S9 derived from Aroclor 1254 induced male Chbb:Thom (Wistar) rat liver, produced in-house		
Media:	Overnight culture: 100 ml Standard I Nutrient Broth inoculated by one colony of corresponding master plate or 500 µl frozen permanent culture and incubated at 37 °C in a shaking incubator. For R-factor strains, ampicillin (0.025 mg/ml) added. S. Typh. TA 102 inoculated only with 500 µl of frozen permanent culture and incubated overnight in the presence of 1 µg tetracycline per 100 ml nutrient broth. Plating: Merckoplate Minimal-Glucose-Agar used for all strains except TA 102, for which Minimal-Glucose plates were self-plated using DIFCO Agar, and 2.0 ml of Top agar solution		
Strains:	*Salmonella typhimurium*: TA 98, TA 100, TA 102, TA 1535, TA 1537 *Escherichia coli*: WP2 uvrA pkM101		
Quality Assurance (40 CFR §160.12):	Included, acceptable		

Summary: 1. **Rating:** Non-mutagenic

2. **Classification:** Acceptable

Procedure: Range-finding assay, with and without activation, used test material at 5 - 5,000 µg/plate at half-log intervals. Final test series included 50, 158, 500, 1580 and 5,000 µg per plate. The positive controls were strain-specific, and the test decision criteria encompassed in the study was presented in the study report.

Results: None of the experiments using the test material or negative/solvent controls showed any significant increase in bacterial mutants. However, all positive controls were strongly mutagenic.

BPB's Comment: This study meets the requirements of §84-2 and indicates the test material does not produce bacterial mutation in a variety of *Salmonella typhimurium* or *Escherichia coli* cells either with or without exogenous activation.

DATA EVALUATION REVIEW FOR
MAMMALIAN CELL (V79) GENE MUTATION TEST (§84-2)

Product Manager:	90	**Reviewer:**	Carol Frazer, Ph.D.
MRID No.:	442979-05	**Report Date:**	June 26, 1996
Testing Laboratory:	Institute of Toxicology, University of Mainz		
Report No.:	AFP 128		
Author(s):	Prof. Dr. Oesch, Dr. Hengstler		
Test Material:	Insect Repellent 3535, Batch K 20961687; colorless liquid		
Cell Source:	Dr. G. Turchi, Instituto di Mutagenesi e Differenziamento, Pisa, Italy		
Solvent Used:	Ethanol (1%)		
Controls:	Negative: Nonactivation -- ethanol (160 µl), dimethylsulfoxide (60 µl), Activation -- ethanol (96 µl), dimethylsulfoxide (60 µl); Positive: Nonactivation -- N-methyl-N'-nitro-N-nitrosoguanidine; Activation -- 7,12-dimethylbenz(a)anthracene		
Activation:	S9 derived from Aroclor 1254 induced male Sprague-Dawley rat liver		
Media:	Dulbecco's modified minimal essential medium, supplemented with 5% fetal calf serum and penicillin/streptomycin (DME-FCS); with S9 mixture, Dulbecco's phosphate buffered saline (PBS) with 20 mM HEPES, pH 7.4		

Quality Assurance (40 CFR §160.12): Included, acceptable

Summary:

1. **Rating:** Non-mutagenic
2. **Classification:** Acceptable

Procedure: Pre-testing by sponsor indicated only the highest dose tested, 5,000 µg/ml, showed weak activity, but no levels were affected in a toxic mode. However, doses chosen for the first set of further testing ranged from 50 - 5,000 µg/ml. On day 1, 1,500,000 cells seeded per 150 mm Petri dish. On the following day, cells exposed to test compound in the presence (24 hours) and absence (3 hours) of S9 mixture, after which the cells washed and maintained for 8 days. After removal of test compound and washing with PBS, cultures maintained until day 8 with 30 ml normal DME-FCS with one subculture on day 5. Cells harvested by trypsinization and replated at density 1,000,000 cells/petri dish containing 6-thioguanine (7 µg/ml) in 6 replicate plates or 100 cells/plate without 6-thioguanine (3 replicates) for cloning efficiency. Plates were fixed and stained after 8 days (cloning efficiency), or 10-11 days (6-thioguanine). Criteria for assay acceptance presented in study report. Positive and negative controls plated for both activated and nonactivated series.

Results: Cells treated with test substance without activation showed similar mutation frequencies as the negative control. The mean mutation frequencies in one series were primarily less than 10×10^{-6}, but at 500 µg/ml, the frequency increased to 5.7 fold above the mean (10.95×10^{-6}). The mean of the positive control series hit a minimum of 300×10^{-6}.

In test compound-treated activated cells, the highest mutation frequency was 4.03×10^{-6}, while the positive control minimum was 275×10^{-6}.

BPB's Comment: This study meets the requirements of §84-2 and indicates the test material does not produce gene mutations in V79 cells.

DATA EVALUATION REVIEW FOR MUTAGENICITY TEST
CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY CELLS (§84-2)

Product Manager:	90	**Reviewer:**	Carol Frazer, Ph.D.
MRID No.:	442708-30	**Report Date:**	November 25, 1996
Testing Laboratory:	Corning Hazleton Inc.	**Report No.:**	17982-0-437
Author(s):	Hemalatha Murli, Ph.D.		
Test Material:	IR3535, Lot #K20961687 WIL-3017B#1; clear colorless liquid		
Cell Source:	Dr. S. Wolff, University of California at San Francisco, California		
Solvent Used:	Ethanol (1%)		
Control Materials:	Negative: cells in culture medium, with and without S9 Positive: Nonactivation -- mitomycin C (0.04 and 0.08 µg/ml); Activation -- cyclophosphamide (10.0 and 15.0 µg/ml)		
Activation:	S9 derived from Aroclor 1254 induced male Sprague-dawley rat liver (Molecular Toxicology, Inc., Lot # 0667)		
Media:	McCoy's 5A culture medium supplemented with 10% fetal bovine serum, 1% glutamine, and 1% penicillin and streptomycin, at approximately 37°C, in an atmosphere of about 5% CO_2 in air		
Quality Assurance (40 CFR §160.12):	Included, acceptable		

Summary:

1. **Rating:** Non-mutagenic
2. **Classification:** Acceptable

Procedure: For range-finding assay, with and without activation, test article dissolved in ethanol, at 502 mg/ml and concentrations tested ranged from 0.167 - 5020 µg/ml at a dosing volume of 1% (10.0 µl/ml). Cultures were incubated for 24 hours with 5-bromo-2'-deoxyuridine to enable the majority of cells to progress through about 2 cell divisions. Replicate cultures of negative control, solvent control, 2 doses of positive control, as well as various concentrations of the test material tested: nonactivated -- 250 - 3,000 µg/ml for 28 hours; activated -- 500 - 5,000 µg/ml for 3 hours. All cultures harvested at 30 hours from initiation of treatment. Further testing on activated cultures at doses of 1,000 - 4990 with monitoring of pH included. Cultures examined for chromosomal aberrations.

Results: In the range-finding assay, complete cytotoxicity was observed at 5020 mg/ml in the nonactivation assay, and severe cell delay at 1670 with cell cycle delay persisting in 167 and 502 in the nonactivation assay and in 5020 in the activated series. No significant increase in chromosomal aberrations present in any of the nonactivated series, but at 4,000 and 5,000 µg/ml of the activated series, such an increase was seen ($p \leq 0.01$). In the repeat series, the two highest doses, 3,990 and 4,990, also demonstrated increased aberrations at the same statistical significance. However, these concentrations were also shown to produce cytotoxic effects to the cells.

BPB's Comment: This study meets the requirements of §84-2 and indicates the test material does not produce chromosomal aberrations in Chinese hamster ovary cells except with exogenous activation and at toxic levels.

DATA EVALUATION REVIEW FOR INDUCTION OF MICRONUCLEI
IN THE BONE MARROW OF TREATED MICE (§84-2)

Product Manager:	90	**Reviewer:**	Carol Frazer, Ph.D.
MRID No.:	442708-31	**Report Date:**	September 1996
Testing Laboratory:	Corning Hazleton Inc.	**Report No.:**	221/12-1052
Author(s):	R. Marshall		
Species:	CD-1 out-bred mice		
Weight:	males: 19-29 g, females: 19-25 g		
Age:	males: 30-41 days, females: 30-41 days		
Sex:	77 males, 77 females: 4 range-finding and 3 test groups		
Source:	Charles River UK Ltd., Margate, UK		
Test Material:	IR3535, batch number K20961687 WIL-3017B#1; liquid		
Control Materials:	Negative/vehicle: miglyol 810 neutral oil Positive: cyclophosphamide (2 mg/ml in physiologic saline)		
Quality Assurance (40 CFR §160.12):	Included, acceptable		

Summary: 1. **Rating:** Non-mutagenic

2. **Classification:** Acceptable

Procedure: Animals acclimated for 1 day for range-finding and 5 days for main study. For range-finding assay, 3/sex followed for 4 days at 1373 - 5000 mg/kg, evaluating clinical signs of toxicity and weight gain/loss. Main study used 5 groups: vehicle; 3 doses of IR3535: 475, 950, and 1,900 mg/kg; and 40 mg/kg cyclophosphamide as a positive control. Fed animals weighed before dosing and concentrations calculated based on a dose volume of 20 ml/kg for intraperitoneal dosage. Test article and vehicle-treated mice killed in groups, 24, 48 and 72 hours after treatment; positive controls killed 24 hours after treatment. Femurs from each animal were removed and bone marrow extracted for examination following staining. Slides examined "blind". Both polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) were initially counted up to at least 1000 cells; then counting continued until at least 2000 PCE were observed and recorded. The vernier coordinates of all cells containing micronuclei were recorded to a maximum of six per 2000 cells scored. Acceptance criteria included in study report.

Results: At the two highest doses of the range-finding study, all animals died. At the next lowest dose, 2113 mg/kg, lethargy, prostration, unsteady gait, piloerection, tremors, irregular breathing, eye closure, convulsion and gasping occurred, but only on the day of dosing. Subsequently, no signs observed. At 1373 mg/kg, lethargy and unsteady gait, but again, only on the day of dosing.

The high dose in the main study, 1,900 was calculated to be about 73% of the LD_{50}, and close to the MTD. No statistically significant increases in PCE/NCE ratios when compared with vehicle controls at any time. Positive controls indicated the laboratory was efficient.

BPB's Comment: This study meets the requirements of §84-2 and indicates IR3535 does not induce micronuclei in the polychromatic erythrocytes in the mouse bone marrow.

DATA EVALUATION REVIEW FOR 28-DAY TOXICOKINETIC STUDY WITH DERMAL APPLICATION TO RATS (§85-1)

Product Manager:	90	**Reviewer**:	Carol Frazer, Ph.D.
MRID No.:	442708-33	**Report Date**:	02-JUL-1996
Testing Laboratory:	RCC, Research & Consulting Company, Ltd. and BRL, Biological Research Laboratories, Ltd.		
Report No.:	398823		
Author(s):	G. Arcelin and Dr. D. Stegehuis		
Species:	HanIbm : WIST (SPF) rat		
Weight:	males: 195.6 - 216.6 g, females: 193.6 - 212.7 g		
Age:	males: 8 weeks, females: 10 weeks		
Sex:	36 males, 36 females: 3 test groups		
Source:	BRL, Biological Research Laboratories Ltd.		
Test Material:	Insect Repellent 3535, batch number K 20961687; faintly yellowish liquid		

Quality Assurance (40 CFR §160.12): Included, acceptable

Summary: 1. **NOAEL(mg/kg/day)**: 3,000

2. **Classification**: Unacceptable

Procedure: Study performed as a preliminary for 90-day dermal toxicity study. Animals acclimated 1 week before study. Rats shaved approximately 24 hours before dosing and then on a weekly basis during the study. Because the purpose was to decide dose groups for the 90-day study, no vehicle control group tested. Test material was provided to laboratory in 2%, 20% and 60% mixtures, respectively. In order to have the creams approximately equal in consistency, it was necessary to alter the vehicles. The same additional constituents were in all creams, but in varying doses; the 60% IR3535 had still another.

Dosing mixtures (100, 1,000, 3,000 mg/kg/day) at 5 ml/kg applied evenly over skin as uniformly as possible, covered with an occlusive dressing wrapped around the abdomen and fixed with an elastic adhesive bandage for 6 hours. Some animals managed to remove the occlusive dressing (between 8-25% of subjects in each test group), but study continued as animals would be exposed on subsequent days. After removing covering, rats cleaned off with lukewarm tap water and dried with disposable paper towel. Clinical signs recorded daily, and body weights taken every day before dosing. Blood samples for toxicokinetic evaluation taken twice per animal, on days 3 and 28. During testing days, samples from 3/12/group taken pre-dosing, 1 hr, 3 hr and 6 hr postdosing. Blood was pooled by sex and time period. Necropsy performed on all subjects.

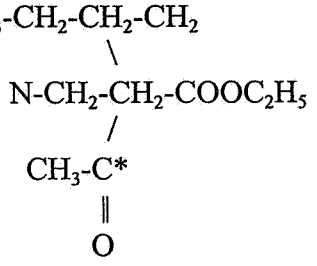
Results: One male from the lowest dosing group died after blood sampling on day 3. No clinical signs of systemic toxicity noted in any group, and behavior and appearance were normal in all animals throughout the study. Body weight gain and mean body weights were similar in all

groups and within the normal range for rats of this strain and age. Most animals of the low and mid dose and all animals of the high dose groups showed local skin reactions in the application area. The most frequent findings were patchy erythema (grades 1 and 2) and scaling. Incidence, persistence and severity were dose-dependent. A few more severe skin reactions seen in all dose groups, one female demonstrating slight necrosis on the last 6 days of treatment and 1 male at the middle dose with slight epidermal lesions during the last 10 days of treatment. Toxicokinetic measurement indicated carboxylic acid metabolite of the test substance in plasma of all treated animals every time sampled after dosing, but predosing concentrations were too low to determine. At the low and mid dose peak concentration measured at 1 hour postdosing, but at the high dose, with the exception of males on day 28, peak concentration was at 3 hours. Concentrations higher on day 3 than on day 28 in all groups.

BPB's Comment: This study indicates the test material is not a subacute dermal toxicant in the rat following 28 days of treatment.

DATA EVALUATION REVIEW FOR BIORETENTION STUDY IN MALE RATS AFTER DERMAL ADMINISTRATION OF THE C^{14}-LABELLED COMPOUND AT A DOSE LEVEL OF 1.0 MG/CM^2 (§85-3)

Product Manager: 90 **Reviewer**: Carol Frazer, Ph.D.
MRID No.: 442708-34 **Report Date**: April 9, 1996
Testing Laboratory: RCC UMWELTCHEMIE AG
Report No.: 612966
Author(s): Dr. R. Burri
Species: Wistar, KFM-WIST, outbred, SPF-quality and BDIX/Hanlbm(SPF), pigmented rats
Weight: 175-210 g
Age: 7 weeks
Sex: 20 males
Source: BRL, Biological Research Laboratories Ltd., Switzerland
Test Material: C^{14}-labelled IR3535, lot no. K20961687; liquid
Chemical structure:



Quality Assurance (40 CFR §160.12): Included, acceptable

Classification: Unacceptable

Procedure: Animals acclimated 5-6 days before study (**only males tested, not females**). All animals received 1.0 mg/cm^2 of radioactive test material in an application volume of 10 mg/10 cm^2/100 mg cream (10% IR3535 in cream base similar to that used in 28-day dermal toxicity test). [It is interesting to note the formulation for treatment was based on the fact the commercial formulation had 20 mg/100 mg cream. Why the lower dose for checking bioretention?] After application, animals sacrificed at 1, 4, 8, 24 and 72 hours (exposure only 24 hours). Test material applied dermally in a cream following anaesthesia (1-2 hrs) to ensure proper dosage and handling of the test material for a maximum of 24 hours exposure. About 24 hours prior to treatment, all rats clipped free of hair over back and sides and area wiped with acetone. After treatment, application dose allowed to dry on skin about 30 minutes. A gauze cover (~15-20 cm^2) fixed with occlusive adhesive bandage outside the treated area to protect the area (except for 1 hour exposure). Animals remained anaesthetized during the entire application period. Bandage removed from skin after exposure and before sacrifice. Skin washed 3 times with soap-solution and once with water via gauze patches. Gauze patches, rinse and bandages retained for radioactivity determination. Food and water provided *ad libitum*. All animals necropsied following sacrifice, body weights recorded, and blood and tissue samples taken. Blood samples

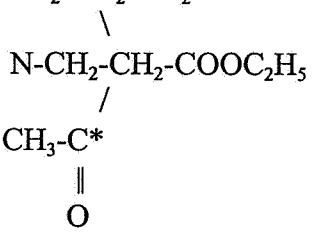
digested and analyzed for radioactivity in duplicate. Radioactivity in blood measured by liquid scintillation of plasma samples. Organ samples digested in duplicate as far as possible and radioactivity determined. Gauze patches for washing and bandages were extracted twice and subsamples determined for radioacticvity. Protocol for test reported in study.

Results: This dermal study demonstrated that about 50% of IR3535, applied to skin at 1,030 $\mu g/cm^2$, was absorbed in a time-dependent manner during dermal exposure. Highest radioactivity levels were found in the treated skin, liver and kidney. After 24 hours, radioactivity levels decreased, indicating efficient elimination of the test article. Only skin and liver still had measurable levels at 72 hours. Radioactivity in all organs except skin and liver was highest at 1 hour of exposure. In liver, radioactivity increased up to 8 hours and subsequently decreased. In all other organs/tissues measured, values were maximally 0.02% for all time intervals. The total amount of radioactivity in organs/tissues (excluding treated skin) was 0.81% at 1 hour and this decreased to 0.60% at 24 hours. No differences noted between pigmented and albino rats.

BPB's Comment: This study is not meant to meet the requirements of §85-3, being a simple bioretention study in a single sex, but it does indicate IR3535 is fairly rapidly excreted, and not substantially retained in the male rat.

DATA EVALUATION REVIEW FOR DERMAL ABSORPTION AND PHARMACOKINETIC STUDY ON VARIOUS ORGANS AND TISSUES OF MALE RATS AND EXCRETION PATTERN OF RADIOACTIVITY AFTER SINGLE DERMAL ADMINISTRATION OF THE C^{14}-LABELLED COMPOUND (§85-3)

Product Manager: 90 **Reviewer:** Carol Frazer, Ph.D.
MRID No.: 442708-35 **Report Date:** March 25, 1996
Testing Laboratory: RCC UMWELTCHEMIE AG
Report No.: 398147
Author(s): Dr. R. Burri
Species: Wistar, KFM-WIST, outbred, SPF-quality rat
Weight: 170-220 g
Age: 7 weeks
Sex: 86 males
Source: BRL, Biological Research Laboratories Ltd., Switzerland
Test Material: C^{14}-labelled IR3535, lot no. K20961687; liquid
Chemical structure:



Quality Assurance (40 CFR §160.12): Included, acceptable

Classification: Unacceptable

Procedure: Animals acclimated 5 days before study, including 1 day in metabolism cage. All animals received radioactive test material in an application volume of 10 mg/10 cm^2/100 mg cream at 3 dose levels (**only males tested, not females**). These levels (0.1, 1.0 and 10% IR3535 in cream base similar to that used in 28-day dermal toxicity test) provided a final dose level of 0.01, 0.1 and 1.0 mg/cm^2. [It is interesting to note the formulation for treatment was based on the fact the commercial formulation had 20 mg/100 mg cream. Why the lower dose for checking pharmacokinetics?] After application, animals sacrificed at 1, 4, 8, 24 and 72 hours (exposure only 24 hours). This was applied dermally in a cream formulation following anaesthesia (1-2 hrs) to ensure proper dosage and handling of the test material for a maximum of 24 hours exposure. About 24 hours prior to treatment, all rats clipped free of hair over back and sides and area wiped with acetone. After treatment, application dose allowed to dry on skin about 30 minutes. A gauze cover (~15-20 cm^2) fixed with occlusive adhesive bandage outside the treated area to protect the treatment (except for 0.5 and 1 hour exposures). These animals remained anaesthetized during the whole application period. Bandage was removed from the skin after exposure and before sacrifice. Animal's skin washed 3 times with soap-solution and once with water with gauze patches. Gauze patches, rinse and bandages retained for radioactivity

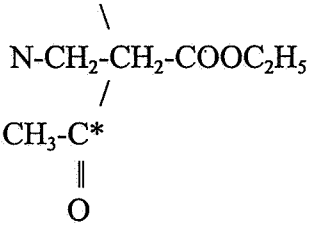
determination. Food and water provided *ad libitum*. Excretion measured through urine and feces **(however, no expired air)**. Urine collected in flasks and sampled at sacrifice, at 10 hours (24 and 72 hour animals) and in 24 hour time intervals after beginning of treatment (72 hour animals). When available, urine in bladder was collected at sacrifice and added to the last urine sample. Feces collected and sampled at sacrifice and in 24 hour time intervals after beginning of treatment (72 hour animals). Feces lyophilized, homogenized and aliquots used for chemical analysis. All animals necropsied following sacrifice, body weights recorded, and blood and tissue samples taken. Blood samples digested and duplicates analyzed for radioactivity. Radioactivity in blood measured by liquid scintillation of plasma samples. Organ samples digested in duplicate as far as possible and radioactivity determined. Gauze patches for washing and bandages were extracted twice and subsamples determined for radioactivity. Protocol for test reported in study. **(No metabolites identified in carcass, urine or feces.)**

Results: This dermal study demonstrated significant absorption of IR3535, in the dose range of about 10 to 1,000 $\mu g/cm^2$. At the low and middle dose levels, absorbed radioactivity slightly increased during the first 10 hours after dermal exposure and remained the same from 10 to 24 hours. The absorption rate continuously decreased from 0.5 to 24 hours and excretion occurred mainly via urine. At the highest dose level, a time-dependent increase in the amount absorbed occurred from 0.5 to 24 hours, but between 0.5 and 10 hours absorbed radioactivity was lower compared to the other dose levels, indicating saturation of absorption mechanism. Absorption rate at 24 hours 10 times higher than for the middle dose reflecting 10 times the dose, but absorption rates from 0.5 to 10 hours only about 3 to 7 times higher, again indicating saturation of absorption process. Highest radioactivity levels were found in the treated skin, liver and kidney, and at the highest dose level in the intestinal tract. From 24 to 72 hours, radioactivity levels decreased, and by 72 hours, excretion of radioactivity almost complete, but still on-going. Radioactivity efficiently eliminated via urine.

BPB's Comment: This study is not meant to meet the requirements of §85-3, being a simple pharmacokinetics study in one sex, but it indicates IR3535 is fairly rapidly excreted and not substantially retained in the male rat (although percentage in expired air not determined).

DATA EVALUATION REVIEW OF THE PHARMACOKINETICS AND METABOLISM STUDY AFTER INTRAVENOUS AND DERMAL APPLICATION OF THE C^{14}-LABELLED COMPOUND TO MALE RATS AND RABBITS (§§85-1, 3)

Product Manager: 90 **Reviewer**: Carol Frazer, Ph.D.
MRID No.: 442979-06 **Report Date**: August 7, 1996
Testing Laboratory: RCC UMWELTCHEMIE AG
Report No.: 392883
Author(s): Dr. A. Van Dijk
Species: Wistar, KFM-WIST, outbred, SPF-quality rat and New Zealand White: CRL: KBL (NZW) BR
Weight: rats: 191-217 g; rabbits: 1.7-2.0 kg
Age: rats: 7-9 weeks; rabbits: ~3 months
Sex: rats: 18 males; rabbits: 4 males
Source: BRL, Biological Research Laboratories Ltd., Switzerland
Test Material: C^{14}-labelled IR3535, lot no. K20961687; liquid
Chemical structure:



Quality Assurance (40 CFR §160.12): Included, acceptable

Classification: Unacceptable

Procedure: Animals acclimated at least 5 days before study, including 1-3 days in glass or stainless-steel metabolism cages (**only males tested, not females**). All animals received radioactive test material either via intravenous injection (1.04 mg/ml in ethanol/saline, ~30 µCi/animal; 10 rats [tail], 2 rabbits [ear]) or dermally (1011 mg/ml in ethanol, 50 µl [~150 µCi/animal]; 8 rats, 2 rabbits) applied directly to the shaven dorsal skin (4 cm^2) via a Hamilton syringe. Animals placed in metabolism cages immediately after injections or dermal application, and blood samples taken from injected animals at 0.5, 1, 2, 4 and 96 hours. The injection site was pressed by a gauze patch which was kept for radioactive analysis. After the dermal application, samples taken at 1, 4, 8 and 24 hours. Blood samples taken directly from the rats heart at sacrifice (2 killed at each sampling time), and from rabbits through the ear vein. Radioactivity in blood measured by liquid scintillation of plasma samples. Excretion measured through urine and feces collected (**no expired air however**), urine into flasks and collected at 24, 48, 72 and 96 hours following injection and at the 24 hour period following dermal application. Radioactivity of urine determined by liquid scintillation of subsamples, while feces lyophilized, homogenized and combusted. About 48 hours prior to dermal treatment, all rabbits clipped free of hair over back and sides and area wiped with acetone, and at 24 hours prior to treatment, rats treated identically. Application followed anaesthesia (1-2 hrs) to ensure proper dosage and

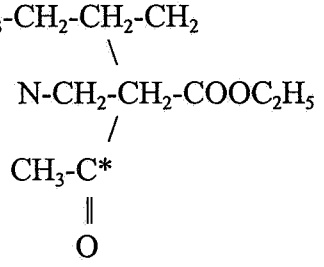
handling of the test material for a maximum of 24 hours exposure. A gauze cover (~15-20 cm^2) fixed with occlusive adhesive bandage outside the treated area was placed on rats to protect the treatment area, and for rabbits, a whole body stocking. Bandage was removed from the skin after exposure and before sacrifice. Animal's skin washed 3 times with soap-solution and once with water with gauze patches. Gauze patches, rinse and bandages retained for radioactivity determination. Food and water provided *ad libitum*. All animals sacrificed and body weights recorded. Gauze patches pressed following injection extracted twice with bidistilled water and measured for radioactivity **(however, not dermal bandages)**. High performance liquid chromatography (HPLC) used as method analysis for characterization of parent compound and metabolites. **(No examination for metabolites in organs or carcass.)** Protocol for test reported in study.

Results: This study demonstrated significant excretion of IR3535 following both intravenous and dermal absorption. After iv injection, plasma levels hit a peak at 0.5 hour and decreased rapidly, with an excretion half-life in both species of 0.5-0.7 hours. Recovery in the urine was 95.9% and feces 16.8% at 96 hours with most excretion in first 24 hours. Following dermal absorption, plasma values were highest at 4-8 hours, with 18% absorbed in the rat and 27% absorbed in the rabbit. Based on the 24 hour excretion in urine and feces, radioactivity dermally absorbed amounted to about 8% in rat and 18-26% in rabbits. In no case was parent compound detected in any plasma sample, and almost all radioactivity was detected in the acid of IR3535, indicating rapid ester hydrolysis.

BPB's Comment: This study is not meant to meet the requirements of either §§85-1 or 3, being a simple pharmacokinetics and metabolism study in a single sex, but it does indicate IR3535 is rapidly excreted and not substantially retained in the male rat or rabbit (although percentage in expired air not determined).

DATA EVALUATION REVIEW OF *IN VITRO* METABOLISM
IN HEPATOCYTES OF RAT AND MAN (§85-1)

Product Manager: 90 **Reviewer**: Carol Frazer, Ph.D.
MRID No.: 442979-07 **Report Date**: March 8, 1996
Testing Laboratory: Ludwig-Maximilians-Universität Munchen, Germany; Merck KGaA
Report No.: 16/34/95
Author(s): Dr. Bernhard J. Ladstetter
Species: Lewis rat; human tissue from subject undergoing resection because of liver metastasis, no liver disease apart from tumor, no drug history known
Weight: rats: 200-230 g; human: not
Age: rats: not given; human male: 62 years old
Sex: male
Source: not given
Test Material: C^{14}-labelled IR3535, lot no. K20961687; (this batch given as liquid in other studies)
Chemical structure:



Quality Assurance (40 CFR §160.12): Was not designed to meet GLP requirements; preliminary study of metabolic comparability in rat and man, no QA

Classification: Unacceptable

Procedure: Hepatocytes isolated from tissue and cultured in Dulbecco's Modified Eagle Medium supplemented with insulin, hydrocortisone, gentamycin and fetal calf serum, and further cultured by collagen gel immobilization for at least 4 days. Test material added to cells on culture day 4, and subsequently incubated for 2, 4, 8 and 24 hours. At these time points, culture spun off cells and stored until metabolites measured by gradient high pressure liquid chromatography (HPLC), and metabolites confirmed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Degradation of test sample checked by incubating in culture without cells.

Results: This study demonstrated that *in vitro* metabolism in hepatocytes from both rat and man was practically identical. Both species rapidly metabolize IR3535 to the same carboxylic acid metabolite, as found after *in vivo* metabolism in laboratory animals. No unchanged test substance present in any sample.

BPB's Comment: This study is not meant to meet the requirements of §85-1, being a simple comparative hepatocyte metabolism study in two species, but it does indicate IR3535 is rapidly metabolized in both the human and rat male in the same manner, and that the rat is an appropriate model for human.

DATA EVALUATION REVIEW OF THE
LABORATORY EVALUATION FOR THE
EFFICACY OF MERCK KGaA, INSECT REPELLENT 3535
AGAINST STABLE FLIES AND DEER TICKS (§95-9)

Product Manager: 90 **Reviewer**: Carol Frazer, Ph.D.
MRID No.: 442708-36 **Report Date**: April 19, 1996
Testing Laboratory: Mulrennan Research Laboratory, Florida A & M University
Report No.: IR3535-FL-95.01
Author(s): James E. Cilek, Ph.D.
Species: nymphal *Ixodes scapularis*, adult *Stomoxys calcitrans*
Test Material: IR3535, batch number K20962087; physical constitution not reported
Control Materials: Positive: DEET
Quality Assurance (40 CFR §160.12): Study executed as a range-finding study

Procedure: Protocols for studies not provided in study report. Assays with nymphal deer ticks were performed both *in vitro* and *in vivo*. The *in vitro* assays consisted of 2 hour exposure intervals for each chemical/concentration at 1 mg/cm^2 on filter paper. *In vivo* assays used the middle index finger technique at a rate of 1 mg/cm^2 for up to 8 hours. The stable fly test utilized treated forearms (rate of application 1 mg/cm^2) in an *in vivo* assay for 2 hours.

Results: IR3535 repellency ranged from 0 to 8.9%, and the DEET repellency, even at the highest concentration (60%), was not significantly higher in the *in vitro* assay. However, the middle index finger technique indicated significant repellency of IR3535 against both nymphal *Ixodes scapularis* and adult *Stomoxys calcitrans*. Less than 90% repellency of deer ticks was noted for IR3535 at all concentrations one hour after treatment and continued to decline thereafter for 4 hours. At time of treatment, IR3535 and DEET were not significantly different in repelling ticks at 5% and 15% compared with DEET at 15% and 60% respectively. Although IR3535 at 10% achieved more than 93% repellency during this time, repellency of DEET at 30% was slightly better than IR3535. Repellency decreased with both IR3535 and DEET at the lower concentrations after 4 hours, but at 30% IR3535 and 60% DEET, repellency lasted at nearly 100% for up to 8 hours.

BPB's Comment: Efficacy studies are not required for a manufacturing use product. Nevertheless, the test material is an efficacious repellent against nymphal deer ticks (it does not evaluate efficacy against other tick stages) and stable flies.

DATA EVALUATION REVIEW OF THE *IN VITRO* ASSAY TO DETERMINE THE EFFICACY OF MERCK KGaA, INSECT REPELLENT 3535 AGAINST BLACK FLIES, DEER FLIES AND STABLE FLIES (§95-9)

Product Manager: 90 **Reviewer:** Carol Frazer, Ph.D.
MRID No.: 442708-37 **Report Date:** June-October, 1995
Testing Laboratory: Wildlife Research Station, Algonquin, Ontario and Trent University, Peterborough, Ontario
Report No.: IR3535-CN-95.02
Author(s): Fiona F. Hunter, Ph.D. and James F. Sutcliffe, Ph.D.
Species: adult *Simulium venustum* complex (878), *Tabanidae* (772) , and *Stomoxys calcitrans* (440)
Source: Black and deer flies collected in the wild, while stable flies were obtained from an in-house colony at Trent University
Test Material: IR3535, batch number K20962087; physical constitution not reported
Control Materials: Positive: DEET; Negative: Blank; Vehicle: ETOH
Quality Assurance (40 CFR §160.12): Study executed as a range-finding study

Procedure: *In vitro* assays with black flies, deer flies and stable flies were performed by measuring the insect landing rates, time spent on membranes and probing of repellent-treated membranes occurred. With black flies, only probe numbers used in calculations of efficacy, while with deer and stable flies, the landings and time spent on treated membranes measured efficacy. Ethanol-treaated membranes used as negative controls.

Black flies were held in 20 mL collection vials with fine mesh bottoms placed on latex condom membranes stretched over the surface of a 2" x 4" metal box, equipped with a heating element and thermostat to maintain a constant 37°C. Deer and stable flies transferred from glass scintillation vials and placed individually in a plexiglass cylinder (~7.5 cm in diameter and 15 cm high) which rested on a mirror. The top of the cylinder was covered with a fine mesh through which the flies could bite, and this was covered with the treated membranes and weighed down with a pre-warmed glass beaker. A light was trained from above to attract the flies. Membranes were heated on a metal slide warmer, equipped with a heating element and thermostat set at 40°C.

IR3535 tested in several concentrations: 5 for black flies, 1.875 - 30%, 8 for deer flies, 0.9 - 30%, and 5 for stable flies, 15 - 100%. DEET concentrations were 30% for black and stable flies and for deer flies, 30 and 80%. Vehicle control (100 µl ETOH) was the same for all tests. With black flies, an extra negative control, an untreated membrane, was added to the tests. Time of post-application testing exposure for black flies reached a maximum of 5-6 hours at 30% IR3535 and 2-3 hours at 3.75% (just looking for probing); while time allowed for exposure to treated membrane was 120 seconds for deer and stable flies (landing and time spent on membrane).

Results: Black flies: concentrations of IR3535 $\geq$ 3.75% had the same repellency as 30% DEET. Deer flies: 15% IR3535 is equivalent to 80% DEET, while higher concentrations of IR3535 are

significantly better than DEET. Stable flies: somewhat less responsive to IR3535 in that 50% IR3535 is no more effective than 30% DEET, and lower concentrations are less effective. However, higher concentrations of IR3535 are more effective than 30% DEET.

BPB's Comment: The test material is an efficacious repellent against black, deer and stable flies, but the product is not being marketed as an end-use product, but rather as a manufacturing use product, so efficacy studies not required.

DATA EVALUATION REVIEW OF *IN VITRO* ASSAY OF MERCK KGaA INSECT REPELLENT 3535 AGAINST NYMPHAL DEER TICKS, *IXODES SCAPULARIS* (§95-9)

Product Manager: 90 **Reviewer**: Carol Frazer, Ph.D.
MRID No.: 442708-38 **Report Date**: Not provided
Testing Laboratory: Benzon Research
Report No.: IR3535-PA-95.03
Author(s): Gary L. Benzon, Ph.D.
Species: nymphal deer ticks, *Ixodes scapularis*
Test Material: technical grade IR3535
Control Materials: Positive: DEET; Vehicle: Ethanol
Quality Assurance (40 CFR §160.12): Study executed as a range-finding study

Procedure: Assays with nymphal deer ticks were performed by applying dilutions of materials in ethanol (IR3535: 5, 10, 15%; DEET: 15, 30, 60%) evenly to filter paper at 10 mg/cm^2, placing the disks in an uncovered petri dish and a clean disk of aluminum on the center of each treated paper. Ticks were randomly selected and placed in groups of 10 into 15 ml tubes, lightly anaesthetized with CO_2 and transferred to the aluminum disk. One minute was allowed for ticks to recover from the anaesthetization before timing began. At 5, 10, 15, 20, 60 and 120 minutes, the total number of ticks that left the aluminum disk were counted and recorded. Ticks reaching the outer edge of the filter paper disk were removed. Ticks that remained on the aluminum disk and appeared moribund or dead were excluded from the assay and the starting N reduced accordingly. One replicate of this assay consisted on one plate for each concentration plus one control plate containing ethanol only. Assay was replicated 5 times using all new materials, except for aluminum disks which were cleaned with acetone and reused, and new groups of ticks. Scoring system reported in study report.

Results: Fifteen percent IR3535 repelled deer ticks at least as effectively as 15 or 30% DEET in this study. There was a significant treatment effect with all treatment concentrations at all time intervals except 120 minutes. Vehicle control ticks crawled off the aluminum disks quickly and without apparent hesitation, while ticks in containers with repellent-containing paper touched the treated paper and quickly withdrew onto the aluminum disks. They eventually left the aluminum disks, but the rate of dispersion was significantly slowed. That positive control, DEET, has a toxic effect on ticks was apparent in this study. With the DEET-treated paper, many ticks were moribund or dead at 120 minutes. Toxicity was not clearly evident with IR3535.

BPB's Comment: This efficacy study is not required for registration, as the product is a manufacturing use product, not an end-use product. Nevertheless, the test material is an efficacious repellent against nymphal deer ticks, although not tested against tick larvae or adult forms.

DATA EVALUATION REVIEW OF ADDITIONAL EFFICACY STUDIES (§95-9)

Product Manager: 90 **Reviewer:** Carol Frazer, Ph.D.
MRID No.: 442708-40 **Report Date:** Not provided
Author(s): Susan D. Phillips, M.S.
Quality Assurance (40 CFR §160.12): GLP and QA not performed

Efficacy Studies on Insect Repellent 3535

1. Efficacy against the mosquitos *Anopheles gambiae* and *Anopheles funestus*

   At 2 locations in Liberia, legs of 6 healthy volunteers (i.e., 72 subjects in all), were coated with 2 ml of 25% aqueous alcoholic Insect Repellent 3535 (test) and 2 ml ethanol (control) between the knee and ankle, for 6 nights. The number of insects per leg and hour were caught and counted over a period of 6 hours. The trial was carried out under medical supervision, in villages situated near rivers, between 1900 and 0700 hours. Insect Repellent 3535 demonstrated a strong repellent action against the 2 species of mosquitos and 4% of other blood-sucking fly species. Even after 6 hours, 92% of the approaching insects were repelled.

2. Efficacy against the biting fly *Aedes aegypti*

   Forearms of test subjects (n=10) were treated with 0.1 ml/100 cm^2 insect Repellent 3535 at 3 different concentrations (10, 20, 30%)(**vehicle not given**), and held inside cage with 500 free-flying mosquitos for 5 minutes once each hour. The second bite on the forearm triggered the conclusion of this experiment. Duration of repellency dependent on concentration, with 20 and 30% proving almost equally repellent at ~447 minutes. The 10% concentration gave about 252 minutes. [**This write-up seems ambiguous in that the line on the graph has 4 dots, at 10, 15, 20 and 30%, with numbers listed next to the dots at 10, 15 and 20%, but not 30%. However, the text of the experiment did not mention a dose level of 15% tested. Is the spot at 15% on the line in reality the number for 20% and the numbers and dot for 20% in reality 30%?**]

3. Comparison of the efficacy of repellent 3535 and DEET against *Aedes aegypti*

   The Institute for Parasitology of the Veterinary Department of Hannover University showed approximately equal protection between 33% DEET (Tm = 6 h 18 min) and 30% Insect Repellent 3535 (Tm = 7 h 36 min) on 12 volunteers. [**No information on type of test performed or vehicle used.**]

4. Comparison of the efficacy of repellent 3535 and DEET against *Aedes albopictus*

   Dainihon Jochugiku Research Laboratory performed field studies on 2 groups of 4 volunteers with 2.5 and 5% aerosol formulations (**vehicle not given**) of agents applied to right arm of

each subject and left remained untreated as a control. Both repellents were equally effective; there were no bites with the 2.5% concentration for up to 6 hours. Exposure time was 10 minutes/hour, and the control arms received an average of 131 bites.

5. Effectiveness against lice *in vitro*

Specially bred body lice *Pediculus humanus*; strain *Orstrom*, Bondy, used to evaluate the effectiveness of Insect Repellent 3535 against lice. A louse was placed on a 1" tissue square treated with repellent, which was surrounded by 5 rings providing a total of 7 measurement areas. Trial carried out at room temperature under artificial light on batches of adult lice.

Immediate repellency

Insect repellent 3535 examined under prophylactic usage conditions in 3 separate trials with 3 different batches of lice of different ages on treated fabric, and movement from the treated center measured at 0, 0.5, 2, 8 and 32 minutes. Identical test without treatment used for control. A total of 96 lice over 3 trials placed on tissue soaked with 20% repellent solution in ethanol. 94% of lice fled from the treated center and 71% reached the outer ring of the target. Only 23% of the population remained in the other rings.

Long term repellency

A similar test was performed with fabric soaked 2 days previously in repellent. 90% of the lice left the tissue within 32 minutes after being placed on it. The repellent action also persisted as 71% of the remaining lice were in the outer ring.

6. Effectiveness against lice *in vivo*

Insect repellent 3535, DEET and control compared for efficacy against reinfestation with headlice in children. All groups washed with insecticidal shampoo on day 0. Agents applied as 20% solutions in 95% ethanol on day 0 and 4 of study. Efficacy of either treatment validated on day 7. Before treatment, average of 27 headlice per child. By day 7, the control group had rapidly reinfested and had reached 50% of pretreatment value, whereas the two agents largely prevented reinfestation (DEET slightly less effective than IR3535).

Repellent action of Insect Repellent 3535 against *Ixodes ricinus* ticks (Acari: Ixodiae)
Alicja Buczek, Krysztof Siuda, Ryszard Sebesta; Department of Biology and Parasitology
Silesian Medical Academy, Medyów 18, 40-754; Katowice, Poland

Three solutions of Insect Repellent 3535 (0, 10, 20, 30%) in 50% EtOH were tested for efficacy on the rabbit against adult ticks, especially females. Shaved skin on the rabbit back was treated with ~1 ml/100 cm^2 test substance. After 30 minutes drying, ticks transferred to skin. A group of 10 female ticks used for each of 3 replicates of 10 and 20%, and 2 replicates of 30%. Efficacy measured by numbers of ticks probing skin after 1, 2, 3 and 4 hours. Treatment with 10% solution repels 100% of female ticks through 4 hours. After 20%, repellency similar in magnitude to 10% solution. At 10 and 20% solutions, no mortality of

females occurred, but at 30%, dead ticks were observed starting at 3 hours (5%) and increased at 4 hours (10%).

Comparative efficacy study of two repellents on wasps and honeybees
C. Roubier; Laboratoire BIO-TOX, S.A.R.L.; Sophia Antipolis, Zone de Vallauris; 06220 VALLAURIS

A comparison of Insect Repellent 3535 at 5 and 15% to equivalent concentrations of DEET and a control. Insects exposed to sweet and fruity liquid mixtures outdoors in vessels for 2 weeks to accustom them. Then traps containing the same liquid were placed, but the insects had to pass through a section smeared with the repellent. Captives counted every 24 hours and released, and liquid and repellent replaced every 48 hours. Test continued for 7 days. IR3535 is more effective at repelling wasps and bees than equivalent concentrations of DEET, but both are significantly better (by about 3-fold) than the negative control.

DATA EVALUATION REVIEW OF EVALUATION OF A NATURALLY OCCURRING SUBSTANCE (COMPOUND A) AS A REPELLENT AGAINST MOSQUITOS (§95-9)

Product Manager: 90
Testing Laboratory: ICR, Inc.
Report No.: 059-0051-A
Author(s): Niketas Spero
Species: *Aedes aegypti*
Test Material: NB 7286-41-#4
Quality Assurance (40 CFR §160.12): Study discontinued near beginning, so GLP and QA not performed

Reviewer: Carol Frazer, Ph.D.
Report Date: January 13, 1997

This study seems to have been aborted by Avon, and it is unclear why it was included in this package. The test material was not identified in the study report as IR3535, and the experiment was dropped when participants complained of burning and itching from the sample treatments (whether or not they were IR3535).

Supplemental Studies

Supplemental studies were also submitted: Chemistry Information (MRID 442979-01), Supplemental Dermal Irritation and Sensitization Information on Rabbits, Guinea Pigs and Humans (MRID 442708-23), 4-Week Toxicity of BE 3535 in Sprague-Dawley Rats After Administration in the Diet (MRID 442708-24), Investigatory Study T 9385 with Oral Administration to Rabbits (MRID 442708-27), Investigatory Study T 9400 with Oral Administration to Himalayan and New Zealand White Rabbits (MRID 442708-28), and Toxicology Summary and U.S. Army Hazard Evaluation (MRID 442708-32). They are frequently older studies, and/or were not performed using GLP, or they were not required by FIFRA guidelines. An additional study was submitted, not labeled Supplemental, a Two-week Repeated Dose Toxicity Study in Non-pregnant Rabbits (MRID 442708-39), performed before the developmental toxicity study.

DATA EVALUATION REVIEW FOR SUPPLEMENTAL DERMAL IRRITATION AND SENSITIZATION INFORMATION ON RABBITS, GUINEA PIGS AND HUMANS I (§81-5)

Product Manager: 90 **Reviewer:** Carol Frazer, Ph.D.
MRID No.: 442708-23 **Report Date:** May 7, 1973
Testing Laboratory: Laboratorium für Pharmakologie und Toxicologie
Report No.: Supplemental Dermal Irritation and Sensitization Information on Rabbits, Guinea Pigs and Humans; Supplemental to Guideline §81-5 and §81-6: Local Tolerance Test of Different Preparations of BE 3767 and of BE 3535 in Rabbits (Patch Test)
Author(s): Dr. F. Leutschner
Species: New Zealand White rabbit
Weight: 2.3-2.8 kg
Age: ??
Sex: 3 males, 3 females: intact; 3 males, 3 females: abraded
Source: Bred in-house
Test Material: BE 3535 10% in 50% aqueous ethanol; ??
Quality Assurance (40 CFR §160.12): None -- submitter states material did not meet GLP requirements. However, for this set of experiments, the protocol followed HAZARDOUS Substances, Part 191, Section 11, FDA, Washington 1965

Summary:

1. **Toxicity Category:**

2. **Classification:** Unacceptable

Procedure: No report on acclimation. Rabbits shaved on dorsal area of trunk between fore and hind legs. Test material (amount not given) applied to a linen patch measuring 2.5 x 2.5 cm, then covered with plastic foil, and finally fastened to the animals' backs with a rubberized bandage. After 24 hours exposure, wrappings removed and skin reaction evaluated. In addition, edema measured each week by means of a slide gauge. Observations for erythema made at 1, 24, 48 and 72 hours following patch removal. Draize grading scale used for scoring. Animals observed for behavior, general condition and food consumption and body weights taken daily. Animals followed for 14 days.

Results: This product is not a dermal irritant based on the results of this study. No rabbit in either the intact or abraded groups exhibited any erythema or edema at any point in the study.

BPB's Comment: This study did not provide data which would normally make it acceptable to BPB to complete the requirements for §81-5. Although the introduction to the study indicated behavior, body weights and general condition were observed for each animal daily, the study

report did not include any such information. This information is not required for the study report, but the actual dose used for the irritation test is desired.

DATA EVALUATION REVIEW FOR SUPPLEMENTAL DERMAL IRRITATION AND SENSITIZATION INFORMATION ON RABBITS, GUINEA PIGS AND HUMANS II (§81-5)

Product Manager: 90 **Reviewer:** Carol Frazer, Ph.D.
MRID No.: 442708-23 **Report Date:** July 4, 1979
Testing Laboratory: ??
Report No.: Supplemental Dermal Irritation and Sensitization Information on Rabbits, Guinea Pigs and Humans; Supplemental to Guideline §81-5 and §81-6: Insect Repellent No. 109 d, Skin Care Study in Animals, Tolerance Tests on Human Skin
Author(s): Dr. med. G. Hopf
Species: Pirbright White albino guinea pig, human
Weight: 300-500 g, ??
Age: ??
Sex: 6 males, 6 females; 30 ?
Source: ?, ?, ?
Test Material: INSECT REPELLENT No. 109 d, containing 15% of the active ingredient Repellent BE 3535 and demineralized water, ethanol, Cetiol HE, perfume
Quality Assurance (40 CFR §160.12): None -- submitter states material did not meet GLP requirements. This set, however, was based on the legal requirements for skin tolerance tests of cosmetic agents (LMBG § 24) issued by the Deutsche Gesellschaft für Fettwissenschaft (German Society for Lipid research) with the approval of the Kommission für kosmetische Erzeugnisse (Cosmetics Commission) of the Bundesgesundheitsamt (Federal German Health Office).

Summary: 1. **Toxicity Category:**

2. **Classification:** Unacceptable

Procedure: In guinea pigs, test material (**no amount given**) was applied undiluted daily to shaven area on the flanks and rubbed in for about 30 seconds (**neither occlusive or semi-occlusive coverings applied**). In the human subjects, 12 of which had sensitive skins or suffered from allergies, test material (**no amount given**) was applied to the back and covered with an airtight bandage in a closed epicutaneous patch test (**no time given**) according to Jadassohn and Bloch (**no reference given**). Results read at 24 and 48 hours.

Results: In neither of these studies, did the subjects show any indication of primary dermal irritation.

BPB's Comment: Protocols for the various tests, doses used for treatment and periods of time the test material was in contact with the skin, as usually provided in data reports, were not included in this study report.

DATA EVALUATION REVIEW OF 4-WEEK TOXICITY OF BE 3535 IN SPRAGUE-DAWLEY RATS AFTER ADMINISTRATION IN THE DIET

Product Manager: 90 **Reviewer**: Carol Frazer, Ph.D.
MRID No.: 442708-24 **Report Date**: March 20, 1974
Testing Laboratory: Laboratorium für Pharmakologie und Toxicologie
Report No.: Supplemental to §82-2
Author(s): F. Leushner, A. Leushner, W. Schwedtfeger, W. Dontenwill
Species: Outbred strain of Sprague-Dawley rat
Weight: 100 - 105 g
Age: Males: 38 days; females: 42 days
Sex: 45 males, 45 females
Source: S. Ivanovas GmbH & Co.
Test Material: BE 3535 (lot/batch numbers not included, nor physical state and appearance, although the fact density is reported leads one to believe it is a liquid)
Quality Assurance (40 CFR §160.12): No report on QA and includes a statement that it did not meet GLP

Summary: 1. **NOEL(mg/kg)**: >2,700

2. **Classification**: Unacceptable

Procedure: **(Guideline §82-2 is a Repeated Dose Dermal Toxicity (21-day) in the Rat, Rabbit or Guinea Pig. This study was a 4-week oral toxicity study in the rat. The subchronic oral toxicity guideline for the rat is §82-1.)** A control and two treatment dose groups (15/sex/dose) at 0, 900 and 2700 mg/kg/day **(FIFRA guidelines require 3 treatment groups and a control)** utilized in this 4-week oral study **(§82-1 requires 90 days or 13 weeks)** in the diet. Test material homogeneously mixed with food offered daily to test animals and adjusted weekly for increasing weight and decreasing food consumption as animals aged. Animals observed daily for behavior and general condition and food consumption and weighed weekly. Water consumption and fecal consistency also checked daily. Hematology, clinical chemistry and urinalysis measured in 10 animals/sex/group before initiation of dosing and after the four weeks of treatment. After treatment, all animals' eyes were examined using a hand slit-lamp, and, in addition, a simple noise test was performed and the rats' teeth were examined. All animals necropsied, organs weighed and histopathology conducted.

Results: No deaths occurred. No clinical signs, no behavior modification, food and water consumption and body weight gain were within normal ranges. None of the hematological, clinical chemistry or urinalysis parameters measured were outside the normal range and eyes, hearing and teeth were free from pathological changes and histopathology normal.

DATA EVALUATION REVIEW OF INVESTIGATORY STUDIES
ON RABBITS FOR DEVELOPMENTAL TOXICITY

Merck KGaA submitted two supplemental investigative studies on IR3535 with oral administration to different strains of rabbits, effects on body temperature and histopathology in the non-pregnant Himalayan rabbit (MRID 442708-27) and plasma half-life and toxicity of IR3535 on non-pregnant rabbits of Himalayan and New Zealand White strains (MRID 442708-28).

On November 15, 1995, the Study Director of the Institute of Toxicology of Merck KGaA signed a protocol to evaluate whether Insect Repellent 3535 (IR3535) affected body temperature, laboratory values or histopathology of non-pregnant female Himalayan strain rabbits. The final report signed March 11, 1997 (MRID 442708-27). Because of earlier studies which observed decreases in body temperatures following a dose of 1 g/kg/day in both pregnant and non-pregnant Himalayan rabbits, lower doses were evaluated. In two groups of five Himalayan rabbits, at doses of 0.1 g/kg/day and 0.3 g/kg/day for 10 days, no mortality was seen. No changes in food consumption, body weight gain appeared normal except for brief loss of weight at dosing, no alterations in hematologic readings, no significant clinical signs observed. No reduction in body temperature was noted, and changes in blood chemistry were minor. 2/5 rabbits in each dose group developed gastro-mucous membrane hemorrhages. Historical rate of spontaneous activity not known, but acidification of drinking water in the laboratory may have contributed to this result.

A further study was performed by Merck KGaA (MRID 442708-28), with a protocol signature on January 10, 1997 and final report signed on March 11, 1997. Because of the difference in results between two developmental toxicity studies performed in the Himalayan and New Zealand White rabbit strains, this investigatory study concentrated on a comparison in plasma levels between the two rabbit strains. Three female rabbits of each strain received 0.6 g/kg/day Insect Repellent 3535 for 10 days. Peak levels in the blood were between ½ hour and 1 hour following administration on day 1 and 10. The chemical was cleared from the blood within 24 hours (checked after days 1 and 10) and no accumulation was noted. No difference observed between plasma half-lives. No mortality, and body weight gain appeared normal after initial weight loss similar to that seen in the other investigatory study. Histopathologic examination of the rabbits revealed atrophy of mucous membranes in 2 Himalayan and 1 New Zealand White animal, as noted in previous study.

Each study appeared to be conducted adequately to support conclusions by the authors that Insect Repellent 3535 did not cause a decrease in body temperature in the non-pregnant Himalayan rabbit at lower doses and that plasma levels of the chemical in the two strains evaluated were not different in the non-pregnant animals at the higher dose tested. However, neither of these provided any reason as to why the developmental study in the Himalayan rabbit could not be utilized.

DATA EVALUATION REVIEW OF TOXICOLOGY SUMMARY
AND U.S. ARMY HAZARD EVALUATION:
SUPPLEMENTAL TO GUIDELINE SERIES §81, §82, §83 AND §84

MRID 442708-32 includes several observation categories conducted over the period of 1972 through 1986 (several studies submitted separately in a complete form), as follows:

acute toxicity
- oral
- dermal
- phototoxicity

primary irritation
- eye
- dermal

skin sensitization
photosensitization
longer-term toxicity
- oral (gavage)
- oral (diet)
- dermal

genotoxicity
developmental toxicity

A review of the U.S. Army's conclusion on the use of this repellent (December 1976-April 1977) was also attached.

Acute toxicity of BE 3535 after oral administration to rats

5M and 5F/group Sprague-Dawley rats given undiluted test material by gavage at 7.9, 10.0, 12.6, 15.9 and 20.0 ml/kg. Sedation, reduced food consumption, ataxia, abdominal and lateral position, coma observed. Death occurred within 6 to 24 hours during coma. Surviving animals recovered within 48 hours. The LD_{50} was 14.0 (12.7-15.4) ml/kg given orally. Necropsy of the animals that died revealed pale, parenchymatous organs. Surviving animals sacrificed at 7 days without pathological findings.

Acute toxicity of BE 3535 after administration to mongrel dogs (pilot study)

1M and 1F/group administered undiluted test material by gavage at 1000, 2000, 4000 and 8000 mg/kg body weight, immediately followed by a dose of 10 ml/kg water. No deaths observed. At 2000 mg/kg and above, vomiting after 30 to 60 minutes, followed by salivation for 20 to 30 minutes.

Acute toxicity of BE 3535 after local application to 1/10 of the body surface of rats

Undiluted test material applied to skin of Sprague-Dawley rats, at 6.35, 7.90 and 10.00 ml/kg (5M,5F/group) (**no covering?**) for 6 hrs, then rinsed and swabbed. No systemic reactions observed, but grade 2 erythema in one rat.

Acute toxicity of BE 3535 after local application to 1/10 of the body surface of mice (pilot study)

Undiluted test material applied to skin of NMRI mice and covered with 5 layers of gauze, aluminum foil and a cuff at 6.35, 7.90 and 10.00 ml/kg (5M,5F/group). Exposure 6-hours after which animals rinsed and swabbed. No systemic reactions observed, however, erythema seen at all dose levels up to grade 3 on first day; most receded to grade 1 by second day. Mild erythema seen on day 3 in 3 M and 2 F at highest dose. No necrosis or edema observed.

Acute toxicity of BE 3535 after local application to 1/10 of the body surface of beagle dogs (pilot study)

Undiluted test article applied to skin of pure-bred beagle dogs, covered with 5 layers of gauze, aluminum foil and a cuff at doses of 6.35, 7.90, 10.00 ml/kg (1M,1F/group). Animals exposed 6-hours, then rinsed and swabbed. No systemic reactions observed, but local grade 2 erythema observed from day 1 to 3 at all dose levels. No necrosis or edema.

Local tolerance test of BE 3535 in rabbits (patch test)

Test material (10% solution in 50% aqueous ethanol) applied to shaven skin of New Zealand White rabbits (3M, 3F) on a 2.5 cm^2 linen cloth, covered with plastic foil and fastened to animals backs with elastic bandage for 24 hours. No local or systemic intolerance.

Eye irritation study of BE 3535 in the rabbit

Test material (10% solution in olive oil) instilled in eyes of 5 rabbits and observed for 72 hours. If eyes not rinsed, a clear erythema and mild to clear swelling occurred 1 minute after application. One hour after treatment, mild secretion occurred and, in one case, corneal opacity observed. Three hours after application, the general reactions had only minorly improved, but corneal opacity was seen in <u>all three</u> cases (**Note that <u>five</u> rabbits referred to above**). After 24 hours, only mild erythema and swelling were observed, cornea normal in all animals. After 48 hours, only negligible erythema present which completely receded after 72 hours. If eyes were rinsed, similar symptoms were seen, but eyes cleared after 48 hours.

Topical hazard evaluation program of candidate insect repellent A13-70763-3 (N-n-butyl-N-acetyl) aminoproprionic acid-ethyl ester

Skin irritation studies conducted with New Zealand White rabbits, 0.5 ml technical grade applied for 24 hours to intact and abraded skin of 6 animals. The compound produced neither primary irritation of intact skin nor skin surrounding abrasion.

Eye irritation studies conducted using New Zealand White rabbits, 0.1 ml technical grade applied for 24 hours to one eye each of 6 animals. Compound produced mild injury to cornea and, in addition, some injury to the conjunctiva in all 6 rabbits at 24 hours after application, and in 3/6 7 days after application.

Skin sensitization studies with Hartley guinea pigs. Intradermal injections (0.1 ml of a 0.1% suspension (w/v) of the compound in mixture of propylene glycol and saline) given to 10 animals. Challenge used 0.1% solution but did not produce a sensitization reaction. DNCB used as a positive control produced marked reaction (10/10).

Photochemical skin studies using New Zealand White rabbits with 0.05 ml of a 25% (w/v) solution of the compound and of a 10% (w/v) oil of bergamot solution (positive control) in 95% ethyl alcohol applied to intact skin of 6 rabbits. After application, rabbits exposed to 365 nm UV light for 30 minutes at a distance of 10-15 cm. Under these conditions, test compound did not cause a photochemical irritation reaction, but positive control did.

Skin tolerance tests in animals and man

1. Skin tolerance tests in animals

For five days, undiluted test material applied once daily to shaven skin of 12 albino guinea pigs (6M, 6F) and rubbed in for about 30 seconds. Contralateral area remained untreated. No skin reactions observed under these conditions.

In the same animals, the local application of the test material was extended to 3 weeks (3x5 days, induction phase). After a 4 day break, the test material was rubbed into a previously untreated contralateral, shaven control area on 3 consecutive days (challenge). There was no reaction seen in the control area at the 24 hour or 2 or 3 day readings.

Test material studied for mucosal tolerance eye irritation according to Draize in 8 albino rabbits (4M, 4F). 0.1 ml of undiluted test material instilled into conjunctival sac of rabbits' eye and left to react by closing lids by hand for 1 second, with other eye acting as control. In half the animals, 1 minute after the application, treated eyes were rinsed with 10 to 20 mls of lukewarm saline for 1 minute. In animals treated this way, spontaneous defense reactions were seen. Subsequently, in 2 of the eyes which were not rinsed, erythema and conjunctivitis observed.

2. Skin tolerance tests in man

Undiluted test material studied in the one-sided closed epicutaneous patch test according to the method of Jadassohn and Bloch in 30 human volunteers. Twelve were known to have sensitive skin or to be allergic. Test material applied to backs and covered with an occlusive patch. At 24 and 48 hours, no skin reactions observed. On 10 human volunteers test material

applied for 3 weeks twice a week. After a 12-day break, challenge applied. No reactions over the subsequent 3 days.

Eye irritation study of insect repellents

BE 3535 (20% solution in isopropyl palmitate) studied in 6 rabbits according to EPA guidelines. Test material (0.1 ml) instilled in conjunctival sac of left eye and eye kept closed for 1 second. Eyes not rinsed. Reactions read with a binocular magnifier and a slit lamp at 24, 48 and 72 hours. Untreated right eye served as control. At 24 hours, fluorescein used to examine eyes for corneal injury under a UV lamp with the magnifier and s(p)lit(sic) lamp.

Test material proved to be a weak irritant with all 6 animals displaying a mild erythema and swelling with injected vessels, and extremely mild secretion in 3/6. At 48 and 72 hours after treatment mild irritating effects had disappeared. No corneal effects or iritis seen at any time.

Eye irritation study of BE 3535 (purified) in the rabbit eye

For this study, purified test material used and half the rabbits (3/group) had their eyes rinsed, the other half did not. Erythema and swelling clearly visible in the unrinsed eye, one minute after instillation. Animals all displayed mild corneal opacity, edematous swelling and secretion, purulent to milky. Reactions of intolerance increased up to 5 hours after treatment and then remained more or less constant until 48 hours. At 3 days, reactions decreased, by 7 days, one moderate and one mild corneal opacity were still visible, but, by 8th day these had completely receded. For rinsed eyes, irritation and other effects were less. Irritating effects had completely disappeared by 7 days.

Investigation for phototoxic potential with insect repellent 3535 in albino guinea pigs

Treatment of Himalayan guinea pigs with highest non-irritating concentration (10%) insect repellent 3535 in ethanol with 2% DMSO for phototoxic potential using 8-methoxypsoralen as positive control yielded no evidence of phototoxicity after challenge with UV.

Investigation of photoallergenic potential with insect repellent 3535 in albino guinea pigs

Treatment of Himalayan guinea pigs with highest non-irritating concentration (10%) insect repellent 3535 in ethanol and Freund's complete adjuvant for photoallergenic potential, and using 3,3',4',5-tetrachlorosalicylanilide in acetone over 3 weeks yielded no response to UV-exposure challenge.

Four week toxicity of BE 3535 in sprague-Dawley rats with administration in the diet

15M and 15F/group treated with 0, 900 and 2700 mg/kg/day of test article in diet for 4 weeks. No deaths, no clinical symptoms, physiologic or histological signs that could be treatment-related. NOEL > 2700 mg/kg/day.

Four-week toxicity of the repellent BE 3535 in beagle dogs with administration by gavage

3M and 3F/group pure-bred beagle dogs were treated by gavage daily with insect repellent 3535 in a 1% aqueous methyl hydroxyethyl cellulose gel at 0, 100 and 1800 mg/kg/day for 4 weeks. No dog died. At 100 mg/kg/day no reactions ensured, but at 1800, repeated vomiting occurred in all dogs from 30 minutes after dosing. Vomiting followed by slightly increased salivation. No safe indications of reactions of intolerance were revealed by external, clinical, macroscopic or histopathological examination. The LOAEL (with the exception of vomiting) was assumed to be above 1800 mg/kg/day.

Four-week toxicity of the repellent BE 3535 with administration by gavage to NZW rabbits

3M and 3F/group of pure-bred New Zealand White rabbits were treated by gavage with insect repellent BE 3535 at 0, 500 and 1500 mg/kg/day for 4 weeks. Controls received 1.5 ml of 1% aqueous carboxymethyl cellulose gel/kg. No effects at low dose, but high dose led to deeper breathing and unrest for a short time. Food consumption was reduced and body weight gain also diminished. No other signs or symptoms noted, either macroscopically or microscopically. NOAEL assumed to be between 500 and 1500 mg/kg/day.

Local and general (systemic) tolerance test of BE 3535 with 4 week application to the dorsal skin of NZW rabbits

3M and 3F/group of New Zealand White rabbits were administered insect repellent BE 3535 by topical application to intact dorsal skin (~10% of body surface) at 0, 3.33, 10.00 and 33.33% solutions in 1% aqueous methyl hydroxymethyl cellulose gel 300 P (1 ml/kg/day) for 4 weeks. No deaths occurred. No systemic toxic signs or symptoms other than dermal irritation observed. At the 2 highest doses, erythema and edema developed, dose-dependently. They reached maximum at 14 days and then slowly receded. At end of study, mild to moderate erythemas and swelling could still be observed. No further alterations seen. Histological examination revealed findings of varying intensity consisting of round cell infiltrates, reactive acanthosis with hyperkeratosis and parakeratosis as well as leucocyte infiltration.

Insect repellent 3535: Trial in vitro for mutagenic potential in bacteria with and without addition of a metabolizing system

150, 300, 600, 1200, 2500 and 5000 µg/plate and equivalent amount of preincubation mixture (S-9; male rats; Aroclor 1254) used on *Salmonella typhimurium* TA 100, TA 98, TA 1535, TA 1537, TA 1538 and *Escherichia coli* WP2 uvrA as tester strains. S-Aminoanthracene, 9-aminoacridine, daunomycin, 1-ethyl-2-nitro-3-nitroso-guanidine, methyl methanesulfonate, N-methyl-N-nitro-N-nitrosoguanidine, 2-nitrofluorene and 4-nitro-1,2-phenylene diamine served as positive controls. No mutagenic activity seen at these levels. Higher concentration levels inhibited bacterial growth. Positive controls worked.

An investigation into the possible induction of mutations at the HGPRT locus of Chinese hamster ovary cells by insect repellent 3535

Preliminary range-finding between 0 and 60 µl/ml were tried and doses between 0 and 4.2 µl/ml without activation and 0 - 8.0 µl/ml with activation were used for test. With metabolic activation, pH adjusted half-hourly to 7.5 with 0.2 M NaOH. Positive controls of ethyl methanesulfonate (without activation) and dimethylnitrosomine (with activation) utilized. At the high doses both with (6.0, 7.0 8.0) and without (3.8, 4.2) activation, cells died, but at lower doses, no concentration-dependent increased numbers of mutants were observed. Positive controls prolific.

An investigation of the effect of BE 3535 on the pregnant rabbit and the fetus with administration by gavage (pilot study with one dose level)

Ten New Zealand White rabbits were treated by gavage with 1500 mg BE 3535/kg/day from days 6 through 18 with 10 more for controls (1.5 ml of 1% aqueous methyl hydroxyethyl cellulose gel MH 300 P/kg). Only sign was deeper breathing and unrest among dams for about 20 to 50 minutes after administration. These decreased over time, but were still obvious at end of treatment period. Neither prenatal development, fetal numbers, weights, resorption rate, nor postimplantation loss affected. Placental weights and other fields measured were unremarkable, with no malformations.

An investigation into the effects of BE 3535 on the pregnant rat and the fetus with administration by gavage (pilot study with one dose level)

Twenty pregnant Sprague-Dawley rats were given 1800 mg/kg/day BE 3535 from days 6 through 18 by gavage and an equal number of control animals given 1.8 ml of 1% aqueous methyl hydroxyethyl cellulose gel MH 300 P/kg/day. Unrest in dams for about 1 hour after administration and food consumption temporarily reduced. Prenatal development not affected, nor any other maternal or fetal assessment measured.

Topical hazard evaluation program of candidate insect repellent A13-70763 s[N-n-butyl-N-acetyl] aminoproprionic acid-ethyl ester study no. 51-0014-77 December 1976 - April 1977

The candidate insect repellent, 3-[N-n-butyl-N-acetyl] aminoproprionic acid-ethyl ester, caused mild eye (0.1 ml) irritation but no skin (0.5 ml) or photochemical (25% in ethanol, 365 nm for 30 min) irritation in rabbits. No sensitization (0.1 ml of 0.1% propylene glycol and saline injection) reactions in guinea pigs and did not demonstrate acute ingestion.

DATA EVALUATION REVIEW OF A TWO-WEEK REPEATED DOSE TOXICITY STUDY OF IR3535 IN NON-PREGNANT RABBITS (§83-3)

Product Manager:	90	**Reviewer:**	Carol Frazer, Ph.D.
MRID No.:	442708-39	**Report Date:**	January 6, 1997
Testing Laboratory:	WIL Research Laboratories, Inc.		
Report No.:	WIL-149022		
Author(s):	James L. Schardein, M.S., A.T.S.		
Species:	New Zealand White rabbit		
Weight:	3234 - 4519 g		
Age:	19 - 34 weeks		
Sex:	16 females		
Source:	Hazleton Research Products, Inc., Denver, Pennsylvania		
Test Material:	Insect Repellent 3535, Batch: K20961687; clear colorless liquid		
Quality Assurance (40 CFR §160.12):	Included, acceptable		

Summary: 1. **LOAEL(mg/kg):** >600 mg/kg/day

2. **Classification:** Unacceptable

Procedure: A vehicle (1% carboxymethylcellulose and 0.1% Tween™ 80) control and one treatment (600 mg/kg/day) dose group (6/group) utilized in this 13-day gavage oral study. Dosage volume of 5.0 ml/kg used for both groups. All animals observed daily for overt signs of toxicity, body weights, food consumption and body temperatures. Necropsy with macroscopic examination performed on all rabbits.

Results: All animals survived to scheduled sacrifice. No clinical signs observed and body temperatures unaffected by treatment. Food consumption was however, slightly reduced during the first few days of treatment and subsequent weight gain diminished. No test article administration-related findings noted at necropsy.

BPB's Comment: This study in the female rabbit indicates the test material is not severely toxic at this oral dose and the dose is suitable for a complete developmental toxicity study. In addition, the study highlighted the method of administration, a 12-gauge stainless steel gavage cannula, also considered acceptable.

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Pages 70 through 74 are not included in this copy.

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_____ Identity of product inert ingredients.

__✓__ Identity of product inert impurities.

__✓__ Description of the product manufacturing process.

__✓__ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

_____ FIFRA registration data.

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